16

MEMORANDUM

TO: NDA 50-790 Restasis® (cyclosporine)

FROM: David Roeder, Associate Director of Regulatory Affairs, Office of Drug Evaluation IV DRoeder 12/18/03
Edward Cox, MD, MPH, Acting Director, Office of Drug Evaluation IV
EWC 12/18/03

RE: Review of the Administrative Record Related to the Classification of Antibiotic Drugs Approved for Non-Antimicrobial Indications

DATE: December 18, 2003

QUALIFICATIONS

Mr. Roeder has a Masters Degree in Plant Pathology (specializing in the molecular biology of bacterial plant pathogens). He has five years of experience as a research scientist in laboratories that studied human plasma proteins. Mr. Roeder has worked as a regulatory specialist in the Center for Drug Evaluation and Research (CDER) at the Food and Drug Administration (FDA) for more than 13 years. He spent the first 10 years at the agency as a Regulatory Health Project Manager in the Division of Cardiovascular and Renal Drug Products, and for the past three years he has held the position of Associate Director for Regulatory Affairs in the Office of Drug Evaluation IV (ODE IV). ODE IV has oversight over scientific and regulatory review of investigational new drug applications (INDs) and new drug applications (NDAs) for drug products indicated for antimicrobial use and drug products that are indicated for immosuppressive use (including cyclosporine formulations for systemic use) in recipients of solid organ transplants. The Office of Drug Evaluation V has primary oversight for, among other things, drug products (either antimicrobial or immunosuppressive) that are indicated for dermatologic or ophthalmologic indications or that are marketed over-the-counter.

Dr. Cox's educational background includes a Bachelor of Arts degree in Chemistry from the University of North Carolina at Chapel Hill, a Doctor of Medicine (M.D.) degree from the University of North Carolina School of Medicine, a Master of Public Health degree from the Johns Hopkins University. Dr. Cox completed an internship and residency in Internal Medicine at the Hospital of the University of Pennsylvania in Philadelphia. He then went on to complete a fellowship in Infectious Diseases at the National Institute of Allergy and Infectious Diseases at the National Institutes of Health in Bethesda, Maryland. Dr. Cox practiced clinical infectious diseases for two years. He has been with the FDA for over five years. He has served as a Medical Officer and a Medical Team Leader in the Division of Special Pathogen and Immunologic Drug Products within FDA's CDER. He became Deputy Director of ODE IV within FDA's CDER in February of 2003. He is currently serving as Acting Director of ODE IV. Dr. Cox is certified by the American Board of Internal Medicine in both Internal Medicine and Infectious Disease.

PURPOSE OF THIS REVIEW

The purpose of this document is to summarize the nature and extent of our review of FDA's classification of cyclosporine and other drugs as antibiotic drugs (*i.e.*, drugs that are classified as antibiotic drugs, but are not approved for antimicrobial uses). This group of drugs includes a number of antibiotic drugs approved for the treatment of cancers and immunosuppressive drugs that the FDA has determined meet the statutory definition of "antibiotic drug" under former section 507 of the Federal Food, Drug, and Cosmetic Act (FD&C Act) or current section 201(jj) of the FD&C Act and have therefore been classified as antibiotic drugs by the FDA.

Below we first set forth the documents we examined in performing this review. The next section sets forth the reasons why we believe Dr. Ramsey's 1994 conclusion that cyclosporine is an antibiotic drug substance is based on reasonable factors and a reasonable assessment of those factors. The decision to classify cyclosporine, and therefore Restasis, which contains cyclosporine as its active ingredient, as an antibiotic drug is consistent with our decisions to classify other antibiotic drugs without regard to whether the antibiotic drug is approved for antimicrobial uses. In the final section we briefly cite some of the points with regard to the materials submitted on behalf of Allergan.

DOCUMENTS SEARCHED AND/OR REVIEWED

We reviewed the following:

1. Selected portions of the NDA files for all NDAs for antibiotic drugs for the treatment of cancers and antibiotic drugs that are immunosuppressive that were regulated under former section 507 of the FD&C Act. By "selected portions," we mean all available documents that were generated by the FDA (i.e., reviews, memoranda, letters, minutes of meetings) during the review of the original NDA submission. In cases where there were signals that discussions relating to a drug's classification as an antibiotic may have occurred after approval, the review was expanded to cover that period. This review included the following NDAs:

   Approved Antibiotic Drugs for the Treatment of Cancers

   NDA 50-109: Mithracin (plicamycin)
   NDA 50-443: Blenoxane (bleomycin sulfate)
   NDA 50-450: Mutamycin (mitamycin)
   NDA 50-467: Adriamycin (doxorubicin)
   NDA 50-484: Cerubinine (daunorubicin hydrochloride)
   NDA 50-577: Zanosar (streptozocin)
   NDA ——— Epirubicin
   NDA 50-629: Adriamycin (doxorubicin)
   NDA 50-661: Idamycin (idarubicin hydrochloride)

NDA 50-682: Cosmogen (dactinomycin)
NDA 50-704: Daunoxome (liposomal daunorubicin)
NDA 50-718: Doxil (doxorubicin hydrochloride)
NDA 50-731: Daunorubicin
NDA 50-734: Idamycin (idarubicin injection)
NDA 50-763: Mytozytrex (mitomycin)
NDA 50-778: Ellence (epirubicin)

Approved Antibiotics Drugs that are Immunosuppresive

NDA 50-573: Sandimmune (cyclosporine)
NDS 50-574: Sandimmune (cyclosporine)
NDA 50-625: Sandimmune (cyclosporine)
NDA 50-708: Prograf (tacrolimus)
NDA 50-709: Prograf (tacrolimus)
NDA 50-715: Neoral (cyclosporine)
NDA 50-716: Neoral (cyclosporine)
NDA 50-722: Cellcept (mycophenylate mofitil)
NDA 50-723: Cellcept (mycophenylate mofitil)
NDA 50-735: Neoral (cyclosporine)
NDA 50-736: Neoral (cyclosporine)
NDA 50-737: Neoral (cyclosporine)
NDA 50-738: Neoral (cyclosporine)

2. The December 15, 1994, memorandum from James Ramsey, Ph.D., regarding Sandoz's request for the reclassification of cyclosporine (Attachment 1).

3. The August 1, 1997 memorandum from James Ramsey, Ph.D., regarding the antimicrobial activity of lovastatin and related drugs (Attachment 2).

4. Citizen petition (petition) dated June 13, 2003 submitted by Fish & Richardson P.C., on behalf of Allergan requesting a reclassification of cyclosporine as a non-antibiotic drug. (Attachment 3). Petition for Stay of Action dated August 1, 2003 (attachments omitted). (Attachment 4). Two expert declarations (i.e., Dr. Diane D-S. Tang-Liu, Ph.D. and Dr. H. Dwight Cavanagh, M.D., Ph.D.) submitted by Arnold & Porter by cover letter dated October 24, 2003. (Attachments 5 and 6).

RESULTS

Below we set forth a summary of the results of our search for and review of NDAs for which adequate records could be located. We note that the administrative record for some of the NDAs listed above was not complete. In some of the applications that were approved in the 1960's and 1970's, the documents generated by the FDA prior to and shortly after approval were not available, and could not be located. In other cases, the administrative record spanned the primary time of interest, but decisions related to the classification were not documented in the FDA's administrative record. A number of

records, however, were located that document the approach that was taken by the FDA for these applications with regard to their classification as antibiotic drugs. The relevant information is summarized below.

Approved Antibiotic Drugs for the Treatment of Cancers

- NDA 50-682, Cosmegen (dactinomycin): This NDA was submitted to the agency on October 18, 1962, and approved on December 10, 1964. The established and trade names underwent change prior to the drug's approval. It was referred to as Lyovac, meractinomycin and actinomycin-D in various documents through that period.

  The application was given the NDA number of 14-008 (i.e., not the 50,000 series that are used for antibiotic drugs), which might suggest that it had not been classified as an antibiotic at that time. There is documentation that was generated prior to approval, however, that clearly indicates that it was regulated as an antibiotic drug. A November 7, 1962, internal FDA memorandum documents a discussion of whether dactinomycin should be regulated as an antibiotic. (Attachment 7). An excerpt of this memorandum follows:

  > "Will this NDA come under Section 507 of the Act at all? The drug fulfills the requirements of the definition of an antibiotic as defined in the Act, however, because of its toxicity, its therapeutic use is restricted to antineoplastic action and is not utilized for antibiotic activity in infections.
  >
  > It was suggested that it might be well to keep track of an anti-cancer drug like this by means of certification. It was also pointed out that many other cancer drugs are handled by New Drug procedure.
  >
  > Dr. Grove pointed out that Dr. Lewis and his staff are already over-burdened, and that comments on NDA's submitted might be delayed for a long time.
  >
  > It was decided to submit this question of whether Lyovac should come under 507 or 505 to the Commissioner for a ruling."

  Another document in the Cosmegen NDA file dated August 9, 1962, a chronology of the certification of dactinomycin as an antibiotic, states that it was "transferred to certifiable antibiotic status" on May 1, 1963. (Attachment 8).

  This statement is further supported by a July 10, 1963 internal FDA memorandum that states that "since this drug is the first antibiotic substance to become certified as an antineoplastic agent, it is suggested to follow and evaluate carefully all clinical experience with the marketed drug." (Emphasis in original.) (Attachment 9).

  It was also clear that the policy of classifying a drug such as dactinomycin as an antibiotic was accepted at the highest levels of the FDA. In an August 9, 1963 letter to Senator Milward L. Simpson, the Commissioner of the FDA stated, "you may

wonder why actinomycin-D, intended for use in treating a form of cancer, requires certification as an antibiotic. This arises from the fact that actinomycin-D is produced by a microorganism and is an "antibiotic drug" within the definition of that term as used in the Food, Drug, and Cosmetic Act." (Attachment 10).

It cannot be determined from the administrative record why the application was not given a 50,000 series NDA number. At some point after approval, the NDA number was changed to 50-682. It should be noted, however, that the separate numbering system for antibiotics was devised for administrative purposes.

- NDA 50-484, Cerubidine (daunorubicin hydrochloride): This application was submitted on July 13, 1974 and initially assigned the NDA number ——— On August 7, 1974, a letter was sent to the sponsor acknowledging receipt of its application for batch certification of daunorubicin hydrochloride and stating that the NDA was assigned the number 50-484. Documentation of discussions that may have occurred relating to this change could not be located.

- NDA 50-577, Zanosar (streptozocin): This NDA was submitted on November 10, 1976 and assigned NDA 17-961. It was approved on May 7, 1982 under NDA 17-961. At some point in 1983 (it is not clear from the record), the number was changed to NDA 50-577. Documentation of discussions that may have occurred relating to this change could not be located.

- NDA 50-661, Idamycin (idarubicin): This application was submitted in August 1989 and assigned the number NDA ——— The NDA number was changed to ——— as noted in an August 12, 1989, memo that points to the chemical relatedness of idarubicin to doxorubicin and daunorubicin.

- NDA 50-778, Ellence (epirubicin hydrochloride): This NDA was initially submitted as NDA ——— in July 17, 1984. The agency issued a not approvable letter on July 10, 1985. Pharmacia and Upjohn resubmitted the application on December 15, 1998. This NDA was initially assigned NDA 21-010. After several months, during a "routine administrative screening," the application was reassigned 50-778, thus classifying epirubicin as an "old" antibiotic. The company objected strongly, and met with the agency on August 16, 1999. The meeting minutes dated August 16, 1999 indicate that the sponsor stated that it would apply for designation as an orphan drug, and if that were successful, it would drop its request for reassignment as a non-antibiotic. (Attachment 11). This request for orphan designation was ultimately granted. The product remains classified as an antibiotic drug.

<u>Approved Antibiotics Drugs that are Immunosuppresive</u>

- The first cyclosporine NDAs were submitted to the agency as chemistry pre-submissions on April 22, 1982, and assigned the numbers, NDA ——— and NDA ——. (The regulations allow the pre-submission of the chemistry section to the NDA file for FDA review prior to the submission of the full NDA.) On August 16, 1982,

the review division informed the sponsor that these applications would be regulated as antibiotic drugs, and the applications were reassigned the numbers, NDA 50-573 and 50-574. (Attachment 12). The record does not include a discussion of the rationale for this classification. The full NDAs were submitted on November 12, 1982, and approved on November 14, 1983. There is no indication in the available FDA-generated documents that the sponsor challenged the classification of these drugs as antibiotics during the review or at the time of approval.

- The tacrolimus NDAs were submitted on July 26, 1993 and classified as antibiotic drugs upon the initial submission. They were approved on April 8, 1994. Based on our search, it does not appear that there is any record of the sponsor challenging the classification of tacrolimus as an antibiotic drug.

- The mycophenylate mofitil NDAs were submitted on November 10, 1994 and assigned the numbers NDA —— and NDA —— The acknowledgment letter, however, referred to them as having been submitted under "505(b)/507," indicating that they were recognized as antibiotic drugs. The NDA numbers were later changed to 50-722 and 50-723 during the period in which the drug was being reviewed. They were approved on May 3, 1995, under NDAs 50-722 and 50-723 as antibiotic drugs. Based on our search, it does not appear that there is any record of the sponsor challenging the classification of its drug as an antibiotic.

Memoranda from James Ramsey, Ph. D.

Sandoz challenged in October 1994, the FDA's classification of Sandimmune and Neoral (both containing cyclosporine) as antibiotic drugs. In this challenge, the firm argued that cyclosporine does not have antimicrobial activity in dilute solution. Sandoz proposed a definition of "dilute solution" that would link it exclusively to the minimal inhibitory concentrations (MIC's) of a chemical substance against human pathogens. Apparently, Sandoz's proposal for a limited interpretation of the definition of "dilute solution" would not consider data on antimicrobial effect derived from *in vivo* animal studies or clinical studies in humans.

In response to Sandoz's challenge, Dr. James Ramsey, Supervisory Microbiologist of the Division of Antiviral Drug Products, CDER, FDA, performed a review of the classification of cyclosporine as an antibiotic drug substance. From Dr. Ramsey's review it appears that he considered not only information that Sandoz submitted, but also other relevant information in the literature regarding the antimicrobial activity of cyclosporine, which included both *in vitro* data and *in vivo* animal model data. Dr. Ramsey evaluated the concentrations of cyclosporine that exhibited antimicrobial activity in the available data from *in vitro* studies and *in vivo* animal models of infection. He then linked these data to the concentrations of cyclosporine that are achievable in human plasma when administered at recommended doses. He determined from *in vitro* studies and *in vivo* animal models of infection that cyclosporine has antimicrobial activity against two fungal pathogens, *Cryptococcus neoformans* and *Coccidioides immitis,* at

concentrations that are found in human plasma following the administration of cyclosporine at its recommended doses in patients.

In addition, he found evidence from studies that cyclosporine has antimicrobial activity against HIV when grown in cultured cells in the laboratory and antimicrobial activity in an animal model of malaria infection. With regards to malaria, he notes that there were inadequate pharmacokinetic data available from the animal studies to provide a link between the concentrations at which cyclosporine exhibited antimicrobial activity in the animal model to concentrations that are achievable in human plasma. He then notes that if the cyclosporine levels attained in the animal model of infection for malaria (a mouse model) are similar to what was observed in the animal model for *Cryptococcus neoformans* (a mouse model), the data would suggest that cyclosporine has antimalarial activity at plasma concentrations achievable in human plasma. However, he notes that further evaluation of the relationship of animal drug levels to the levels observed in humans is needed before conclusions can be drawn. Dr. Ramsey also notes that there is also literature on cyclosporine's effect in animal models of parasite infections, beyond the malaria study reviewed within his 1994 review.

Sandoz again challenged the FDA's classification of cyclosporine in March 1997. This time they argued that the cholesterol lowering agents, lovastatin and related drugs, have similar properties to cyclosporine and, by the criteria applied to cyclosporine, should also have been classified as antibiotic drugs. Lovastatin and two other related drugs, simvastatin and pravastatin, are produced by micro-organisms, and they have antimicrobial activity. Dr. Ramsey evaluated the literature to determine if these drugs had antimicrobial activity in dilute solution (*i.e.*, at concentrations found in human tissue when dosed according to approved labeling). The bulk of the memorandum focuses on lovastatin.

Based upon what is described in Dr. Ramsey's memo, he conducted an analysis of the literature to evaluate the data on the antimicrobial activity of lovastatin, simvastatin, and pravastatin. Of the three drugs of interest, only lovastatin and simvastatin were shown to have antimicrobial activity in *in vitro* and *in vivo* animal studies.

The studies that Dr. Ramsey reviewed did not show either lovastatin or simvastatin to have *in vitro* antimicrobial activity at levels found in human tissue in clinical use at the range of approved dosages for lovastatin and simvastatin. In addition, Dr. Ramsey found that most of the *in vitro* studies utilized a growth medium that would enhance the antimicrobial activity of these drugs. The microbes in these *in vitro* studies were grown with severely restricted serum and lipoprotein, conditions that would not be found in human use.

Antimicrobial activity of these drugs in animal models was found to be minimal, and it was not demonstrated that the drug levels at which *in vivo* activity was observed could be achieved in human tissue at approved dosages. Dr. Ramsey concluded that there were inadequate data to support a conclusion that any of these cholesterol lowering agents should be classified as antibiotic drugs.

We believe that, in determining whether a drug substance "has the capacity to inhibit or destroy micro-organisms in dilute solution," Dr. Ramsey's reliance on *in vitro* data and *in vivo* animal data is reasonable for the reasons explained below.

When sponsors conduct adequate and well-controlled clinical studies in humans (*i.e.*, *in vivo* human data), they are generally testing to see whether a drug is safe and effective for a specific indication. Efficacy data from adequate and well-controlled *in vivo* human studies can provide evidence of a drug's clinical efficacy in the treatment of, among other things, an infectious disease.

There are circumstances, however, under which *in vivo* human studies may not demonstrate efficacy in the treatment of a particular type of infection despite the fact that the drug substance has the capacity to inhibit or destroy micro-organisms in dilute solution. For example, the demonstration of clinical efficacy from adequate and well-controlled clinical studies involves a number of factors in living systems that include, among other things, the antimicrobial activity of the drug, whether the drug achieves sufficient concentrations at the site of infection that is being studied, the immune response of the host, the metabolic state of the infecting micro-organism, and the microbial microenvironment. An antimicrobial drug that merely does not achieve adequate concentrations at the site of infection (*e.g.*, an antimicrobial drug that achieves poor concentrations in the bloodstream, or the central nervous system) may have significant microbiologic activity (*i.e.*, the capacity to inhibit or destroy micro-organisms), but may fail to demonstrate clinical efficacy because of inadequate concentrations at the site of infection in the human body. Therefore, reliance upon *in vivo* human data may fail to identify drugs that have the capacity to inhibit or destroy micro-organisms in dilute solution simply because the antimicrobial drug failed to achieve adequate concentrations at the site of infection under study – although the antimicrobial drug substance if evaluated for the treatment of infections at other sites in the body might be found to have clinical efficacy.

The statutory definition of antibiotic drug (under former 507 of the Act and current section 201(jj) of the Act) does not require the demonstration of clinical efficacy in patients with infections, nor does it require data from *in vivo* animal models of infection demonstrating effectiveness. The definition asks whether the drug substance has the capacity to inhibit or destroy micro-organisms in dilute solution.

Data from animal models of infection (*in vivo* animal studies) can provide information on an antimicrobial drug's capacity to inhibit or destroy micro-organisms in a living animal. Like *in vivo* studies in humans, the response in an animal model of infection involves factors other than just the antimicrobial activity of the drug under study, including the ability of the drug to attain therapeutic tissue levels at the site of infection under study, the immune response, the size of the inoculum (large inoculum may lead to an infection that even an effective antimicrobial drug cannot effectively treat), the timing of initiation of antimicrobial therapy, and subsequent dosing. Hence, as is the case for *in vivo* studies in humans, although a finding of antimicrobial effect in an animal model can provide evidence of an antimicrobial drug's capacity to inhibit or destroy micro-organisms, a

negative finding for antimicrobial effect in an animal model does not necessarily exclude the possibility that the drug is an active antimicrobial agent.

The use of *in vitro* testing methods to determine whether a particular micro-organism is inhibited or destroyed by a particular concentration of an antimicrobial drug is one of the cornerstones of clinical microbiology. *In vitro* testing methodologies are typically designed to determine concentrations of an antimicrobial drug that inhibit microbial growth (*e.g.*, the minimal inhibitory concentration for bacterial micro-organisms) or the concentration that destroys micro-organisms (*e.g.*, the minimal bactericidal concentration for bacterial micro-organisms). *In vitro* testing methodologies are not dependent upon many of the complex factors that influence outcomes in infections in animals or humans such as achieving a specific drug concentration at the site of the infection or the host immune response. *In vitro* methods measure the effect of an antimicrobial drug in a less complex system than an *in vivo* animal model or in naturally occurring human infection. *In vitro* methods are dependent upon the techniques used, including factors such as the inoculum size and characteristics of the microbial growth media used. In addition, inhibitory concentrations cannot be determined for all micro-organisms. *In vitro* testing methodologies are important in identifying the antimicrobial activity of drug substances against particular micro-organisms and are relied upon for the selection of antimicrobial therapy every day in hospitals across the United States. *In vitro* testing methods provide information on the capacity of a drug substance to inhibit or destroy the micro-organism being tested.

Results from *in vivo* human studies, *in vivo* animal studies, or *in vitro* studies can provide evidence of the capacity of a drug substance to inhibit or destroy micro-organisms. There are strengths and limitations to each of these approaches for the purposes of measuring the capacity of a drug substance to inhibit or destroy micro-organisms. These limitations are inherent to the biology of the micro-organisms and the settings (*in vivo* human studies, *in vivo* animal studies, or *in vitro* studies) within which the drug is being evaluated.

In summary, evidence of clinical efficacy from *in vivo* human studies can provide evidence of a drug substance's capacity to inhibit or destroy micro-organisms, but a negative result does not necessarily exclude significant antimicrobial activity. The same is true for animal models of infection. Measurement of antimicrobial effect in humans and in animal models is affected by a number of factors. *In vitro* studies can provide information from a system that measures the capacity of the drug substance to inhibit or destroy micro-organisms. Reliance upon data from *in vivo* human studies, animal models of infection, or *in vitro* data can be used to evaluate whether a compound possesses the capacity to inhibit or destroy micro-organisms in dilute solution.

The definition of antibiotic drug does not require the demonstration of clinical efficacy from *in vivo* human studies. Nor does the definition require the demonstration of antimicrobial effect in *in vivo* animal models of infection. The definition of antibiotic drug asks only for demonstration of the drug substance's capacity to inhibit or destroy micro-organisms. The capacity to inhibit or destroy micro-organisms in dilute solution

can be demonstrated using data from *in vivo* human studies, *in vivo* animal studies, or *in vitro* studies. Hence, it is reasonable and appropriate that the agency has relied upon data derived from *in vivo* animal models of infection and *in vitro* data demonstrating the capacity of cyclosporine to inhibit or destroy micro-organisms in dilute solution.

In sum, we believe Dr. Ramsey's 1994 conclusion that cyclosporine is an antibiotic drug substance is based on reasonable factors and a reasonable assessment of those factors.

Materials Submitted on Behalf of Allergan regarding Restasis (cyclosporine)[1]

We have reviewed the materials submitted on behalf of Allergan regarding the classification of Restasis as an antibiotic drug. We have considered the scientific and regulatory information and arguments that are presented in the materials. The materials presented do not change our opinion that cyclosporine is appropriately classified as an antibiotic drug substance. Given the statutory definition of antibiotic drug, it is appropriate for drugs that contain any quantity of the drug substance cyclosporine to be classified as antibiotic drugs. Therefore the classification of Restasis® (cyclosporine) as an antibiotic drug in our opinion is appropriate.

There are a few specific points regarding the classification of cyclosporine, including Restasis, as an antibiotic drug upon which we will comment in this document.

- The classification of Restasis as an antibiotic drug is consistent with the statutory definition of antibiotic drug in that Restasis is intended for human use; Restasis contains a quantity of cyclosporine; cyclosporine is produced by a micro-organism; and cyclosporine has the capacity to inhibit or destroy micro-organisms in dilute solution. The capacity of cyclosporine to inhibit or destroy micro-organisms is clearly described in Dr. Ramsey's 1994 memorandum. Restasis contains cyclosporine and therefore is, in accordance with the definition of antibiotic drug, appropriately classified as an antibiotic drug.

- The definition of antibiotic drug in the FD &C Act does not require that a drug be approved for the treatment of an infectious disease in order to be considered as an antibiotic drug. There are no criteria in the definition of antibiotic drug that speak to the indication for which the drug is approved. As we have shown in this document there are numerous approved antibiotic drugs that are not indicated for the treatment of an infectious disease. We have listed numerous drugs that are appropriately classified as antibiotic drugs that are indicated for the treatment of cancer and several other antibiotic drugs that are immunosuppressant agents.

- The petitioner notes that FDA is limited to the information that it has available on a drug and its antimicrobial activity at the time that a drug is classified as an antibiotic

[1] Citizen petition (petition) dated June 13, 2003 submitted by Fish & Richardson P.C., on behalf of Allergan requesting, among other things, a reclassification of cyclosporine as a non-antibiotic drug. Amendment to the petition dated August 1, 2003. Two expert declarations (i.e., Dr. Tang-Liu and Dr. Cavanagh) submitted by Arnold & Porter by cover letter dated October 24, 2003.

drug. It is true that when the agency decides whether a drug should be classified as an antibiotic drug, the agency can only utilize information and data that are available at that time. (Attachment 8). When available data demonstrate that a drug meets the statutory definition of antibiotic drug, that drug is classified as an antibiotic drug. .

- Allergan also has submitted declarations from Dr. H. Dwight Cavanagh, MD, Ph.D. and Dr. Diane D-S. Tang-Liu, Ph.D. Dr. Cavanagh notes that he has used Restasis in the treatment of his patients and was also involved with the phase III clinical trials for Restasis. He states his familiarity with the scientific ophthalmic literature and that he is not aware of any data on the clinical utility of cyclosporine as an anti-infective. Dr. Tang-Liu notes the absence of detectable blood levels of cyclosporine in patients receiving Restasis, and the lack of data on the local concentrations of cyclosporine with ophthalmic use. She provides her opinion that, given the relatively large size of the cyclosporine A molecule, very little of the drug would penetrate the ocular surface.

  Because the definition of antibiotic drug depends on properties of the drug substance cyclosporine, the declarations are not relevant to the determination of whether Restasis, which contains cyclosporine, is an antibiotic drug.

CONCLUSIONS

Based on our review of the administrative record of the approved antibiotic drugs for the treatment of cancers and drugs that are immunosuppresive drugs, we have found the approach of the agency in its interpretation of the statutory definition of antibiotic drugs to be consistent. Beginning with the anti-cancer drug, dactinomycin, which was submitted to the agency almost concurrent with the enactment of the 1962 amendments (in which the "general" (i.e., non drug-specific) definition of antibiotic drug was established) the agency has interpreted the definition to exclude consideration of the proposed or actual clinical indication of the drug. Since that time, the agency has consistently classified drugs as antibiotic drugs if they met the statutory definition, regardless of their indication. This consistency is demonstrated by the classification of drugs that are used for the treatment of cancers and the immunosuppressive drugs as antibiotic drugs.

The agency has also been consistent in its interpretation of "dilute solution." This term is not defined in the statute. Dr. Ramsey, in addressing Sandoz's (now Novartis's) challenges to the classification of cyclosporine as an antibiotic drug, linked the definition of dilute solution to the actual tissue concentrations that are achieved in humans at approved or proposed dosages. Dr. Ramsey's choice to use the human tissue concentrations achieved based upon considering the range of the drug's approved uses is an appropriate and scientifically reasonable approach.

Attachment 1

MEMORANDUM

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

DATE: December 15, 1994

FROM: James Ramsey, Ph. D.
Supervisory Microbiologist
Division of Antiviral Drug Products

THROUGH: David Feigal, M.D., M.P.H.
Director
Division of Antiviral Drug Products

TO: Murray Lumpkin, M.D.
Deputy Director for Review Management
Center for Drug Evaluation and Research

SUBJECT: Cyclosporine-Request for Reclassification

I have reviewed the data submitted by Sandoz in their submission of October 14, 1994, wherein they responded to CDER's request to provide a scientific basis for the language "in dilute solution" as a criterion for an antibiotic. In this review, I have responded point-by-point to the rationale and discussion (vol. 1, pp.001-004) provided by the sponsor to support their request for reclassification. For clarity purposes in the text provided below, rationale and discussion provided by the sponsor are in bold type, my comments in response are in non-bolded type.

Sandoz:

Sandimmune® (cyclosporine)
NEORAL™ (cyclosporine, microemulsion)
Request for Reclassification

INTRODUCTION

Section 507 (a) of the Federal Food, Drug, and Cosmetic Act defines an antibiotic drug as "any drug intended for use by man containing any quantity of any chemical substance which is produced by a microorganism and has the capacity to inhibit or destroy microorganisms in dilute solution (including the chemically synthesized equivalent of any such substance)."

The key phrase from the above definition is "has the capacity to inhibit or destroy microorganisms in <u>dilute solution</u>" (emphasis added). There have been various interpretations of dilute solution to mean either "in vitro" plate levels or "animal in vivo" plasma or serum levels or "human in vivo" plasma or serum levels. The different interpretations of dilute solution create confusion and may lead to classification of drugs with no clinically relevant antimicrobial activity as antibiotics.

FDA COMMENT:

There are 4 key phrases in the above definition of an antibiotic which are the following:

1) any chemical substance which is produced by a microorganism
2) has the capacity to inhibit or destroy microorganisms
3) in dilute solution
4) including the chemically synthesized equivalent of any such substance.

As will become apparent in the following discussion, the relative importance of all of these key phrases, not just "in dilute solution" are pertinent to the sponsor's request for reclassification and will be referred to where appropriate. Regulations are, by necessity, written in a manner that leaves them subject to broad interpretation. The exclusive focus on a specific or exact definition of circumstances described in regulations often creates more problems than are solved. Consequently, it has always been the policy of this Agency to interpret regulations based upon the collective body of evidence available upon which to make decisions.

Sandoz:

Proposed Definition by Regulation

As a clinically relevant and valid interpretation of "in dilute solution" we propose that minimal inhibitory concentrations (MIC's) of the chemical substance against human pathogens be achievable in human serum, plasma or other relevant body solution (eg, urine) following administration of recommended doses of the drug in the target patient population.

This definition would insure that drugs with in vitro antimicrobial activity only at concentrations that cannot be safely achieved and maintained in man would not be inappropriately classified as antibiotics for human use.

FDA COMMENT:

This argument presupposes that MIC's can be determined for all relevant human pathogens and, furthermore, that clinically relevant antibiotic activity is always highly correlated with patient plasma drug levels approximating MIC values determined in in vitro preclinical assays.

The supposition that MIC's can be determined for all relevant human pathogens is false. Minimum inhibitory concentration is a term appropriately applied to bacterial, fungal and some parasite cell culture assays only. For microorganisms requiring a host cell to support their replication in in vitro cell culture, such as viruses

and some parasites, the terms inhibitory concentration, usually

abbreviated as IC_{50} or IC_{90} (i.e., the concentrations of drug necessary to inhibit growth 50% or 90%, respectively), are used to express drug activity, not MIC values. Furthermore, some microorganisms, such as Mycobacterium leprea, cannot be cultivated in vitro and, therefore, an MIC value cannot be determined.

In addition, the suitability of assay methodologies used in the determination of MIC values is highly relevant to characterization of drug activity. Variations in culture media, organism load, organism strain, incubation conditions, drug exposure time, and experimental design have the capacity to influence MIC values. Acceptance of MIC values without knowledge of how they were determined may satisfy the specific focus on the definition of "inhibition in dilute solution" but knowing how they were determined still requires an evaluation of the collective body of evidence available upon which to make a decision with respect to relevance.

Another concern is that the focus on in vitro MIC's for determining antimicrobial activity completely ignores data from animal model studies. For some microorganisms and for some drugs, in vitro MIC values are less reliable than animal model data for predicting relevant human drug activity. Drug activity in animal studies is usually expressed in terms of effective dose (ED_{50} and ED_{90}) or protective dose (PD_{50} and PD_{90}) and are defined as the drug dose that reduces microorganism load or protects survival in infected animals 50% and 90%, respectively. The terms effective or protective dose are preferred because following drug administration, the drug concentration in the target organ(s) may vary over time or be unknown, depending upon the organ(s) examined. Thus, an MIC value for animal dosing is not a valid parameter to calculate.

The assumption that clinically relevant antibiotic activity will correlate with in vitro MIC values determined for all human pathogens is unwarranted. While MIC values often are predictive of potential human clinical activity, some antibiotics are known to be clinically active against some species of Enterobacteriaceae even though achievable plasma drug concentrations are substantially below the MIC values determined for these microorganisms. On the other hand, it is not uncommon to encounter circumstances where human plasma drug concentration exceeds in vitro MIC values in the absence of clinical efficacy. The reasons for these observed lack of correlations between MIC values and human antibiotic activity are frequently unclear and unpredictable.

Another problem in specifically focusing on the fact that plasma drug concentrations must be equal to in vitro MIC values before one could expect to demonstrate clinically relevant antibiotic activity is that host drug metabolism is not considered. For example,

cyclosporine is extensively metabolized in the host liver and the

parent drug concentration in plasma drops between dosing. However, the plasma concentration of cyclosporine metabolites may rise in some patients and actually exceed the plasma concentration of the parent drug (Sandoz submission Ref 2 - Yee GC, Solomon DR. Cyclosporine. In: Evan WE, Schentag JJ, Jusko WJ, eds. Applied Pharmacokinetics: Principles of Therapeutic Drug Monitoring. 3rd ed. Vancouver, Washington: Applied Therapeutics, Inc; 1992:28-1 - 28-40).

The antimicrobial activities of cyclosporine metabolites have not been adequately characterized although it is known that many retain their immunosuppressive activity. If these metabolites maintain antimicrobial activity as well, matching only parent drug concentration in plasma to preclinical MIC values (determined only for the parent drug) to estimate potential clinical relevance is not valid. Potential clinical antibiotic activity would be the sum of the contributions made by the parent drug and the active metabolites, not just parent drug.

Sandoz:

Classification of Cyclosporine-A: Drug or Antibiotic?

Cyclosporine-A was originally filed as both an oral solution and an intravenous solution in 1982 under the provisions of 505(b) of the Act. The original NDA numbers were 18-773 and 18-772, respectively. A detailed submission chronology for all pending and approved applications is included as Appendix I.

Possibly due to an early publication by Sandoz Pharmaceuticals Division (Ref. 1), which appeared to demonstrate weak antifungal activity in vitro in "dilute solution", these applications were subsequently reclassified as antibiotics (Form 5's 50-574 and 50-573). In addition some animal infection models were studied at extremely high doses (not achievable in man without lethality). It is now clear, however, that maximal plasma concentrations of cyclosporine A, obtained with the highest recommended doses of Sandimmune, do not reach MIC's for any human pathogen for which cyclosporine A has been shown to exhibit in vitro antifungal activity.

FDA COMMENT

The conclusion that animal infection model studies utilized extremely high doses of cyclosporine, not achievable in man without lethality, is premature. Information on bioavailability, pharmacokinetics and pharmacodynamics of cyclosporine in animal species utilized in published studies and how these parameters compare to human circumstances were not addressed by the sponsor. Because of known differences for many drugs with respect to adsorption, distribution, metabolism, and elimination kinetics

among animal species and humans, human equivalent doses for animals often vary substantially when administered on a mg/kg body weight basis. Without including these kind of data in these analyses, correlation of efficacy and toxicity profiles between animal and human studies is less certain (see below). Therefore, without analysis of these parameters, rejection of animal data from being considered in the definition of clinical relevant antibiotic activity, as proposed by Sandoz, is unwarranted.

Sandoz:

In Phase I antibiotic drug development, serum or plasma levels, rather than whole blood levels, of the drug are always evaluated because the serum or plasma is the compartment in which (1) the drug is available to bind to blood borne bacterial or fungal organisms and (2) the drug is available to supply third compartments (e.g., the middle ear). Although drug bound to the cellular elements of blood may be in equilibrium with the plasma and serum, Cmax levels in the plasma or serum are more relevant than in whole blood.

Cyclosporine levels are usually measured in whole blood to reduce variability of the assay, but can also be measured in plasma. Plasma levels of cyclosporine are approximately equal to 40% of whole blood levels (Ref. 2). Since there is little data on serum concentrations of cyclosporine, plasma concentrations are appropriate to assess the antimicrobial activity of cyclosporine.

The highest plasma levels of cyclosporine are obtained during the time immediately prior to and for 1-2 weeks following transplant. Current Sandimmune labeling indicates that the maximum recommended doses are 14-18 mg/kg/day. Peak (Cmax) whole blood cyclosporine levels are generally in the range of 1000-1500 ng/mL (as determined by HPLC) although occasionally levels of 2000 ng/mL are observed (Ref. 2, 3, and Appendix II). Since plasma levels of cyclosporine are 40% of whole blood levels, maximal plasma levels of cyclosporine are in the range of 400-800 ng/mL. Maintenance whole blood levels of cyclosporine are usually below 350 ng/ml consistent with plasma levels of up to 140 ng/ml.

FDA COMMENT

In general, these statements by the sponsor give a balanced opinion of published information relevant to their content. The important aspects of these facts are as follows: 1) there is a difference in blood and plasma cyclosporine levels, 2) concentrations stated are for parent drug and do not include metabolites, 3) peak levels of parent drug are substantially higher than trough levels, 4) assays for the measurement of cyclosporine give variable results (comparisons of results are valid if performed by the same

procedure), 5) bioavailability of oral doses is approximately 30% (range, 5%-90%), 6) therapy is long-term, and 7) the maximum recommended human initial doses are 14-18 mg/kg/day given orally.

Sandoz

Cyclosporine has not been shown to have activity against bacteria. MIC's for common pathogenic bacteria including Streptococcus faecalis, Bacillus subtilis, E. Coli K12, Salmonella typhimurium and Pseudomonas aeruginosa are all over 100,000 ng/mL (Ref. 1) Therefore, maximal achievable plasma levels are over 100 times less than the MIC for any of these potential pathogenic bacteria. Therefore, cyclosporine should not be classified as an anti-bacterial agent.

FDA COMMENT

After review of the data submitted by the sponsor and that retrieved from the National Library of Medicine database by this reviewer, no credible evidence or rationale was identified that would support the conclusion that cyclosporine has any clinically relevant antibacterial activity.

Sandoz

In vitro activity against selected pathogenic fungi has also been reported (Ref. 1 and 4). Table 1 lists the MIC's for these fungal pathogens.

Table 1. MIC's (ng/ml) for Cyclosporine for Fungal Pathogens

Pathogen	MIC	Reference
Saccharomyces cerevisiae	> 100,000	1
Kloekera apiculata	> 100,000	1
Hansenula anomala	> 100,000	1
Pythium debaryanum	> 100,000	1
Rhodoturla rubra	100,000	1
Anixopsis steracoraria	100,000	1
Cospora lactis	31,600	1
Aspergillus flavus	> 10,000	2
Aspergillus fumigatus	> 10,000	2
Candida albicans	> 10,000	2
Candida tropicalis	> 10,000	2
Histoplasma capsulatum	> 10,000	2
Blastomyces dermatidis	> 10,000	2
Neurospora crassa	10,000	1
Trichophyton quickaneum	10,000	1
Aspergillus niger	3,000	1
Curvularia lunata	1,000	1
Coccidioides immitis	1,000	2

As stated above, the maximum plasma concentrations of cyclosporine that may be achieved with recommended doses are in the order of 400-800 ng/mL. Therefore, cyclosporine should also not be classified as an antifungal agent.

FDA COMMENT

The sponsor's conclusion that cyclosporine should not be classified as an antifungal agent, even when using their own proposed definition of "dilute solution", is premature.

The sponsor cited only 2 references in which in vitro determined MIC's for fungal pathogens were reported. Two others, both highly relevant to this report, are summarized below.

Reference 1 - Mody, Christopher H., Galen B. Toews, and Mary F. Lipscomb. 1988. Cyclosporin A Inhibits the Growth of Cryptococcus neoformans in a Murine Model. Infection and Immunity. 56:7-12.

In this study, Mody et al. reported the effect of cyclosporine (Sandimmune IV) on the growth of Cryptococcus neoformans strains 145A, ATCC 36556, and H99 in cell culture and in mice.

For in vitro studies, C. neoformans was cultured for 48 hr in both neopeptone or yeast nitrogen base broth in the presence of cyclosporine at 0.1 or 1.0 ug/ml. Growth of C. neoformans in broth cultures without additives or with Cremaphor-EL (the vehicle for Sandimmune IV) at a concentration equal to that present in the 1.0 ug/ml cyclosporine broth cultures, served as controls. The pH of the culture media with Sandimmune IV, Cremaphor-EL, or without additives was 6.6, 6.7 and 6.2, respectively. Growth inhibition was determined by plating serial 10-fold dilutions of the 48 hr broth cultures onto agar medium and enumerating the number of colony forming units (CFU's) observed after an additional incubation for 48 hr.

Results showed that for strains 145A, ATCC 36556 and H99, 0.1 ug/ml cyclosporine inhibited growth approximately 95, 75 and 98%, respectively; whereas, at 1.0 ug/ml, inhibition was 100% for all strains. Concentrations between 0.1 and 1.0 ug/ml were not evaluated. Similar results were observed with both broth culture media utilized. Growth in media containing Cremaphor-EL and in media without additives was equivalent, suggesting that the pH differences in these cell cultures did not affect fungal growth.

However, many drugs are known to exhibit significantly different antimicrobial activity as a function of pH and blood pH is approximately 7.3. Thus, the possibility exists that cyclosporine

MIC values would be less if evaluated at pH 7.3. Activity determined in mice (see below) at cyclosporine blood concentrations comparable to MIC values shown above would suggest that antifungal activity is maintained at pH 7.3.

These results establish that cyclosporine is fungicidal for C. neoformans in vitro with an MIC value of $\leq$ 1.0 ug/ml. Different types of assays are used to differentiate fungistatic from fungicidal activity of drugs. However, results from this fungicidal assay suggest that cyclosporine fungistatic MIC values for C. neoformans strains could be $\leq$ 0.1 ug/ml.

Mody et al. also evaluated the antifungal activity of cyclosporine against C. neoformans infection in C57BL/6 mice at 20, 50 and 75 mg/kg administered subcutaneously for 7 days. Because cyclosporine administered to mice via this route had not been previously reported, they determined levels of cyclosporine in blood 24 hr after the last dose. Cyclosporine was extracted and quantified by high-performance liquid chromatography (HPLC). Results showed that trough blood levels of 0.30 $\pm$ 0.03, 1.50 $\pm$ 0.10, and 2.75 $\pm$ 0.85 ug/ml were achieved for the above doses, respectively. Corresponding plasma values would be expected to be 0.12, 0.60, and 1.10 ug/ml based upon the observation that plasma cyclosporine concentrations are 40% of blood concentrations. Concentrations of cyclosporine metabolites in mouse blood were not reported.

These results show that trough blood/plasma cyclosporine concentrations in mice following 20 mg/kg subcutaneous injection are comparable to that observed for human transplant patients receiving recommended oral dosing. However, no data were provided to compare peak concentrations between species or to determine concentrations or antimicrobial activities of metabolites present in blood/plasma.

In addition to in vitro studies, Cyclosporine antifungal activity was evaluated by these investigators in mice inoculated intratracheally with C. neoformans. Results obtained with 20 mg/kg s.c. treatment show a highly significant reduction in fungal CFU's within 4 days of treatment (Table 2). Data derived from studies utilizing 50 and 75 mg/kg were not critically reviewed because blood levels produced at these doses were at or above the upper range of levels achievable in humans without inducing severe toxicity. Without additional pharmacokinetics data in mice to compare to human data, assessment of antimicrobial relevance at these higher doses is impaired.

TABLE 2. Effect of cyclosporine on C. neoformans in the lungs of mice after intratracheal inoculation[a].

Cryptococcal strain	CFU (log_{10})/organ in lungs at: Deposition	Day 4	Animal treatment
145A	3.79 ± 0.12	2.60 ± 0.12[b]	Cyclosporine
		4.56 ± 0.05	Control
36556	4.21 ± 0.05	2.62 ± 0.19[b]	Cyclosporine
		4.76 ± 0.11	Control
H99	5.41 ± 0.05	4.92 ± 0.02[b]	Cyclosporine
		5.41 ± 0.06	Control

[a]Mice received Cyclosporine (20 mg/kg per day s.c.) or Cremaphor-EL (control solution) equivalent to 20 mg/kg per day beginning on the day before inoculation. n = 5 in each group.
[b]$P<0.001$.

These results show that the MIC of cyclosporine against C. neoformans, determined in vitro and shown to be active in an infected animal model, is achievable in human plasma following administration of recommended doses of cyclosporine in transplant patient populations.

Reference 2 - Hoeprich, Paul D. and Joanne M. Merry. 1987. Comparative Efficacy of Forphenicinol, Cyclosporine, and Amphotericin B in Experimental Murine Coccidioidomycosis. Diagn. Microbiol. Infec. Dis. 6:287-292.

Hoeprich and Merry, utilizing a broth dilution assay, determined the in vitro MIC and minimum fungicidal concentrations (MFC) of cyclosporine and Amphotericin B against Coccidioides immitis strain Silveria and 10 clinical isolates as shown in the Table 3 below.

Table 3. Susceptibility of Strain Silveria (Geometric Means of Triplicate Determination ± SE) and 10 Clinical Isolates (Geometric means ± SE) of C. immitis was Tested In Vitro Against Cyclosporine and Amphotericin B used to Treat Experimental Murine Coccidioidomycosis.

Drug	C. immitis	MIC (range) ug/ml	MFC (range) ug/ml
Cyclosporine	Silveria	0.3	>20
	10 isolates	0.3 ± 0.04	>20
		(0.15-0.60)	>20
Amphotericin B	Silveria	0.56	NR
	10 isolates	0.56 ± 0.21	>20
		(0.30-2.50)	

NR - not reported

In this study, in vitro MIC values indicated that cyclosporine possessed antifungal activity against C. immitis greater than that

observed for Amphotericin B, an antibiotic drug approved for the treatment of disseminated forms of coccidioidomycosis in human patients. A minimum fungicidal concentration of cyclosporine against C. immitis was not elicited even at concentrations of 20 ug/ml. However, these in vitro results demonstrate that fungistatic MIC values, determined for a laboratory strain and 10 clinical isolates of C. immitis, are achievable in human plasma following administration of recommended doses of cyclosporine in transplant patient populations.

Antifungal activity was also determined in mice injected intratracheally with 100 arthroconidia of strain Silveria. Seventy-two hours after inoculation, groups of 10 mice were intravenously administered the following treatments: a) 0.1 ml of 5% glucose/day for 23 doses (controls); b) cyclosporine at 50, 100, or 200 mg/kg body wt/day for 23 doses; c) Amphotericin B at 0.75 or 1.50 mg/kg body wt on alternate days for 12 doses. Mice surviving at 24 days post-treatment were sacrificed and fungal burden determined in lungs, livers and spleens.

Results obtained showed that survival of controls, 50, 100, and 200 ug/kg cyclosporine, and 0.75 and 1.50 ug/kg Amphotericin B treated mice was 20%, 90%, 60%, 60%, 100% and 100%, respectively. Fungal growth in necropsied tissue from cyclosporine treated mice was slightly less than controls. Cultures of lung tissue from 60% of Amphotericin B treated animals were negative for fungal growth; remaining Amphotericin B treated animals with culture positive lung tissue showed significantly reduced fungal growth.

Survival of cyclosporine treated mice was higher than untreated mice. However, because survival was less at high doses of cyclosporine (100 and 200 mg/kg, survival of 60%) than at the lowest dose evaluated (50 mg/kg, survival of 90%), it is possible that a lower dose with less immunosuppressive activity would prove to have greater benefit but, unfortunately was not evaluated.

Adequate and well controlled human clinical studies for the evaluation of cyclosporine antifungal activity were not found in the literature. Data from published human clinical studies, reporting observations that fungal infections were less/more prevalent in transplant patients or in patients undergoing cyclosporine treatment for autoimmune disease, were insufficient to clearly establish cyclosporine's contribution to changes in fungal prevalence in these patient populations.

Published literature reports relative to potential or actual cyclosporine antifungal activity were scant, but certainly greater than that found for antibacterial activity. Data from these in vitro MIC and in vivo animal studies demonstrated that cyclosporine possessed antifungal activity for at least two human pathogens,

Cryptococcus neoformans and Coccidioides immitis, at concentrations achievable in plasma following administration of recommended doses of the drug in target patient populations. With respect to fungi, there is rationale to classify cyclosporine as an antibiotic, even if the definition of dilute solution as proposed by Sandoz is used.

Sandoz

Cyclosporine has also been reported to exert weak activity against a variety of human parasites (Ref. 5-23) including malaria. However, activity against malaria is only observed at doses of cyclosporine that are nephrotoxic in animals (Ref. 24, 25, and 26). Therefore, Sandoz believes that cyclosporine should be not classified as an antiparasitic agent.

FDA Comment

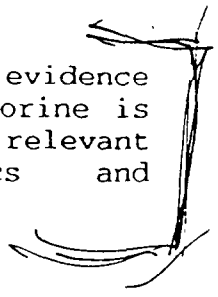
The claim that activity against malaria is only observed at doses of cyclosporine that are nephrotoxic in animals (Ref. 24, 25, and 26) is inaccurate. Nephrotoxicity was only reported in studies with owl monkeys and was thought to be due to the combined effects of malaria and drug toxicities (Ref. 24).

In mice inoculated with Plasmodium yoelii or Plasmodium berghei and administered 25 mg/kg cyclosporine s.c. for 4 consecutive days, parasitemia and death in 15 of 15 and 9 of 10 mice, respectively, was prevented (Ref. 25). Nephrotoxicity was not reported. In an additional experiment, these authors investigated the potential of cyclosporine to cure existing parasitemia produced by P. yoelii (L), P. yoelii (NL), and P. berghei. Two consecutive cyclosporine doses of 25 mg/kg administered s.c. 6 or 8 days after infection was initiated was effective at reducing parasitemia to below detectable levels. However, after 5 days parasitemia reappeared, persisted at relatively low levels for another 5 days and subsequently became undetectable. This pattern was seen even if treatment was extended from 2 days to several weeks except for infections with P. yoelii (NL) in which recrudescence did not re-occur. Resistance to cyclosporine in malaria parasites in animals that relapsed was common. Although not investigated, it would be of interest to determine if combination therapy with 2 or more effective drugs would prevent resistance emergence.

Again bioavailability and pharmacokinetics of cyclosporine was not reported in these studies. However, if blood levels in this study were comparable to those determined in the C. neoformans study described above, these data suggest that cyclosporine has antimalarial activity at plasma cyclosporine levels achievable in transplant patients.

There is a considerable body of literature available on cyclosporine effects in parasite infected animal models, not just

with respect to malaria. Before discounting the weight of evidence from these studies in the effort to determine that cyclosporine is not an antiparasite drug, a comprehensive evaluation of relevant animal and human bioavailability, pharmacokinetics and pharmacodynamics data should be conducted.



Sandoz

Cyclosporine exerts some activity against HIV (Ref. 27). However, approved antiviral drugs are not classified as antibiotics. Therefore, reported antiviral activity of cyclosporine is not relevant to the classification of cyclosporine as an antibiotic.

FDA Comment

The conclusion that reported antiviral activity of cyclosporine is not relevant to the classification of cyclosporine as an antibiotic is incorrect. For example, Vidarabine is a purine nucleoside obtained from fermentation cultures of Streptomyces antibioticus. It possesses in vitro and in vivo antiviral activity against Herpes simplex types 1 and 2, Varicella-Zoster, and Vaccinia viruses. Vidarabine is an FDA approved antibiotic drug indicated for the treatment of acute keratoconjunctivitis and recurrent epithelial keratitis due to Herpes simplex virus types 1 and 2. The sponsor failed to consider all of the 4 key phrases in the definition of an antibiotic drug illustrated in the beginning part of this report. Because Vidarabine, an antiviral drug, meets all of the requisite conditions it is considered an antibiotic drug.

Reference 27 in the Sandoz submission indicates that cyclosporine possesses anti-HIV activity at a concentration of 0.1 ug/ml when added to cell cultures prior to or during acute infection. In addition, numerous reports are in the literature that are relevant to determining cyclosporine antiviral activity. In view of the oversight that antivirals are not classified as antibiotics, the sponsor should consider further the status of cyclosporine's antiviral activity before concluding that cyclosporine antiviral activity is irrelevant to reclassification.

Sandoz

It is also noteworthy that cyclosporine is an immunosuppressive agent and that the risk of some bacterial, fungal and viral infections is increased during cyclosporine therapy (Table 2, Sandimmune[R] package insert, and Ref. 28, 29, and 30). It is also well recognized that most infections occur within the first two to eight weeks after transplant, at the time when cyclosporine levels are highest. This provides further evidence that plasma, blood and tissue levels of cyclosporine obtained during cyclosporine therapy provide no relevant antimicrobial activity.

Table 2. From Approved Sandimmune[R] Package Insert

Infectious Complications in the Randomized Renal Transplant Patients

Complication	Sandimmune[R] Treatment (N = 227) % of Complications	Standard Treatment* (N = 228) % of Complications
Septicemia	5.3	4.8
Abscesses	4.4	5.3
Systemic Fungal Infection	2.2	3.9
Local Fungal Infection	7.5	9.6
Cytomegalovirus	4.8	12.3
Other Viral Infections	15.9	18.2
Urinary Tract Infections	21.1	20.2
Wound and Skin Infections	7.0	10.1
Pneumonia	6.2	9.2

*Some patients also received ALG.

FDA Comment

Reports in the literature suggest that the magnitude of infections in transplant patients may depend upon the level of immunosuppression produced during therapy to prevent organ rejection. The animal model data reviewed above suggest that better antimicrobial activity is achieved at doses that are high enough to elicit antimicrobial effects but low enough that severe immunosuppression is not in evidence. The argument that infections are most severe during early periods following transplantation when dosing of patients is higher is consistent with this observation. Continued research efforts incorporating antimicrobial activities of immunosuppressive drugs may contribute significantly to improvements in clinical care of transplant patients.

CONCLUSIONS:

Credible data to demonstrate that cyclosporine has clinically relevant antibacterial activity was not found in the literature.

Cyclosporine has been shown to possess antifungal activity against 2 relevant human pathogens, Cryptococcus neoformans and

Coccidioides immitis at MIC's achievable in human plasma following administration of recommended doses of the drug in transplant patient populations. Moreover, MIC values for cyclosporine, reported for C. immitis strain Silveria and 10 clinical isolates, were shown to be lower than that determined for Amphotericin B, an antibiotic drug approved for the treatment of disseminated forms of coccidioidomycosis in human patients.

Data in the published literature suggest that cyclosporine has antiviral activity at relevant clinical concentrations. The sponsor discounted these data based upon the incorrect premise that approved antiviral drug products were not classified as antibiotic drugs. These data should be comprehensively evaluated by the sponsor and submitted for review.

Published reports on cyclosporine antiparasite activity are numerous. However, due to several inaccurate assumptions made by the sponsor, the data in this literature was discounted in their response to the FDA request to define "in dilute solution" to support their request for reclassification. To continue the pursuit of this reclassification objective, data relevant to cyclosporine bioavailability, pharmacokinetics and pharmacodynamics in conjunction with efficacy determinations in animals and humans must be comprehensively addressed.

In summary, even if the sponsor's proposed definition of "in dilute solution" is used as the interpretative criterion for cyclosporine reclassification, data are available in the published literature that would support its continued classification as an antibiotic drug.

RECOMMENDATION

Cyclosporine should remain classified as an antibiotic drug.

Attachment 2

MEMORANDUM

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

DATE: August 1, 1997

FROM: James Ramsey, Ph. D.
Microbiology Team Leader
Division of Antiviral Drug Products

THROUGH: Walla Dempsey, Ph. D.
Acting Deputy Division Director
Division of Antiviral Drug Products

THROUGH: Donna Freeman, M. D.
Acting Division Director
Division of Antiviral Drug Products

TO: Murray Lumpkin, M. D
Deputy Center Director for Review Management
Center for Drug Evaluation and Research

SUBJECT: Antimicrobial Activity of Lovastatin and Related Drugs

This report is in response to your request, made during the lovastatin meeting held on June 9, 1997, for an evaluation of the published literature relevant to possible antimicrobial activity of lovastatin and related drugs. I have reviewed the literature relevant to the possible antimicrobial activity associated with the anti-hypercholesterolemia drug, lovastatin. In addition, I have reviewed and included literature reports on antimicrobial activity of related members in the "statin" class of drug products. The database searched for these literature reports was Medline for the years 1962 through June 12, 1997. Other databases have not been searched for information

BACKGROUND:

In the preparation of this report, I have focused on the legal basis for the classification of a drug as an antibiotic drug as defined in Section 507 (a) of the Federal Food Drug, and Cosmetic Act. This legal description defines an antibiotic drug as "... any drug intended for use by man containing any quantity of any chemical substance which is produced by a microorganism and has the capacity to inhibit or destroy microorganisms in dilute solution (including the chemically synthesized equivalent of any such substance)." Therefore, to be determined an antibiotic drug, a human drug must possess the following properties:

1) It must be a drug intended for use by man which is produced by a microorganism or it may be any chemically synthesized equivalent of any such substance.
2) It must have the capacity to inhibit or destroy microorganisms
3) It must demonstrate the capacity to inhibit or destroy microorganisms in dilute solution.

These characteristics of antibiotic drugs have been carefully considered for the purpose of determining if the reported antimicrobial activity of lovastatin and related drug products is sufficient to warrant their reclassification as antibiotic drug products

There are many analogues of "statin" drugs and related chemical substances that exhibit anti-cholesterolemia activity reported in the literature. However, this review will only address antimicrobial activity relevant to "statin" class drug products indicated for anti-hypercholesteremia activity submitted to FDA for marketing approval determinations. Currently, there are six drug products of the "statin" class, indicated for the treatment of hypercholesterolemia, under review or previously approved for marketing by the Center for Drug Evaluation and Research (CDER). Of the six, five, including Mevacor (lovastatin, MK-803, mevinolin, monacolin K), Zocor (MK-733, simvastatin, synvinolin), Pravachol (CS-514, SQ 3100, pravastatin, eptastatin), Lescol (fluvastatin sodium), and Liptor (atorvastatin calcium), have been approved for marketing (Package Inserts Merck, August 31, 1987; Merck, December 23, 1991; Bristol Myers Squibb, October 31, 1991; Sandoz, December 31, 1993; Park Davis, December 17, 1996, respectively). Baycol (cerivastatin sodium tablets) is currently under review (Bayer, NDA 20-740).

Fluvastatin, atorvastatin and cerivastatin are all manufactured by synthetic processes (Package Inserts and NDA 20-740) and as such do not fit the definition component requiring antibiotic drugs to be produced by microorganisms. Therefore, these drug products cannot be classified as antibiotic drugs and, consequently, will not be evaluated for antimicrobial activity in this review. Lovastatin, simvastatin and pravastatin are all drug substances which are either produced by microorganisms or are chemically synthesized equivalents of such substances (Germershaven, et al., 1989; Tobert, 1987; Tsujita, et al., 1986, Sitori, 1990; Alberts, 1988; Alberts, et al., 1980; Alberts, 1990). In addition, all have the capacity to inhibit or destroy microorganisms (*vide infra*). Therefore, all three of these drugs fit the first two definitions required for classification as antibiotic drugs However, the third requirement for antibiotic classification requires that these drugs must demonstrate the capacity to inhibit or destroy microorganisms in dilute solution Interpretation of this requirement is somewhat problematic in that the term dilute solution and the kinds of microorganisms to be inhibited have not been defined. However, there appears to be a consensus within the agency and by some of the regulated drug industry that the microorganisms inhibited should be organisms that are causative agents of human clinical infections. In addition, the term dilute solution has been generally accepted as the drug concentration in preclinical studies that elicits inhibitory activity against microorganisms that correlates with clinically relevant human tissue drug concentrations. Human tissue drug concentrations considered relevant are those that are achieved from doses administered to the human target populations for the indicated use of the drug The data from published literature relevant to interpretation of drug concentrations that "... inhibits in dilute solution ..." are summarized and evaluated in this report

Data on "statin" antimicrobial activity from human studies have not been reported in the literature. Therefore, for this reason, this review contains only antimicrobial activity data generated from *in vitro* cell culture and *in vivo* animal model studies. During the review of these literature reports, it became clear that the preclinical antimicrobial activity data alone were insufficient to permit a rational interpretation of possible antibiotic activity associated with lovastatin and related drug products For example, information on the experimental design of the studies, assays used for determination of activity, and studies on the mechanism of drug action were found to be important parameters when attempting to extrapolate *in vitro* activity results to expected clinical circumstances. In addition, species variability with respect to drug pharmacokinetics, pharmacodynamics, metabolism, elimination, bioavailability, tissue distribution and drug interaction potential were found to be relevant to the interpretations of antibiotic activity potential with respect to the definition "... inhibits in dilute solution ...". Therefore, to the extent possible and within the time-frame available, an effort has been made to provide this information in instances where it was deemed to be of value for the interpretation of parameters relevant to "statin" class drug products' potential antimicrobial activity expression

HISTORY:

In 1971, the Japanese researchers, Akira Endo and Masao Kuroda, began a search for inhibitors of microbial origin that would inhibit the rate limiting enzyme, 3-hydroxy-3-methylglutaryl-Coenzyme A reductase (HMG-CoA reductase), in the biosynthetic pathway for cholesterol (Endo, et al , 1976a; Endo, 1985a; Endo, et al , 1985b; Endo, 1992) They anticipated that certain microorganisms would produce inhibitory products that would interfere with synthesis of required sterols or other isoprenoids required for growth of other microorganisms. They hoped that these products would be effective in inhibiting *de novo* cholesterol biosynthesis and have the potential for reducing plasma cholesterol levels in

hypercholesterolemic humans. By 1973, several compounds that were effective in inhibiting HMG-CoA reductase, including ML-236A, ML-236B (compactin, mevastatin), and ML-236C, had been isolated from cultures of *Penicillium citrinum*. In 1976, after documentation that these inhibitors reduced cholesterol *in vitro* (Endo, et al., 1976a; Kaneko, et al , 1978, Alberts, 1988) and *in vivo* in animal models (Endo, et al , 1976a; Endo, et al., 1992) the first human subjects were treated (reviewed by Endo, 1992; Endo, et al., 1976b; Endo, et al., 1988, Tsujita, et al., 1986). Promising results in lowering plasma cholesterol in these early human studies led to human clinical trials ultimately resulting in the March, 1987, U.S. Food and Drug Administration approval of Mevacor (lovastatin) for the treatment of hypercholesterolemia (Approved Drug Products with Therapeutic Equivalence Evaluations. 14th Ed. 1994. US Department of Health and Human Services, Public Health Service, Food and Drug Administration, Center for Drug Evaluation and Research).

The first publication suggesting that antimicrobial activity was associated with inhibitors of HMG-CoA reductase isolated from fungi appeared in 1976 (Brown, et al., 1976). The authors, citing a reference that was "in preparation", reported that compactin (mevastatin), a potent HMG-CoA reductase inhibitor, was isolated from a culture believed to be *Penicillium brevicompactum* and was detected by its antifungal activity. However, antimicrobial data for the drug (compactin), utilized for the investigations conducted by Brown et al., were not presented in that publication. An intensive computer search of the Medline database for the publication cited "in preparation" was unsuccessful. Evidently, it was never published; thus, a determination regarding the authenticity of the report cited by Brown cannot be made.

The first report of antimicrobial activity attributable to lovastatin was published in August, 1988 (Ikeura, et al., 1988). Thus, although the rationale for the search for these compounds was based upon an antibiotic principle (substance produced by a microorganism that inhibits other microorganisms), at the time of lovastatin's approval by FDA in March, 1987, reports including data on antimicrobial activity of "statin" drugs were not available in the published literature. Consequently, lovastatin was approved as a non-antibiotic drug under Section 505 of the Federal Food, Drug, and Cosmetic Act for its anti-hypercholesterolemia activity. Simvastatin was subsequently approved for its anti-hypercholesterolemia activity in December, 1991. Only three publication were found in the literature on simvastatin antimicrobial activity (Grellier, et al., 1994; Coppens et al., 1995a; Coppens et al., 1995b). Pravastatin was approved for its anti-hypercholesterolemia activity in October, 1991. Antimicrobial activity associated with pravastatin was not found in the literature searches conducted. However, because of structural and mechanism of action similarities to lovastatin and simvastatin, it is predictable that similar levels of antimicrobial activity, as has been reported for the other "statins", may exist for pravastatin.

The question under consideration in this report is the following: now that antimicrobial activity for lovastatin and simvastatin has been reported in the literature, are the published data sufficient to meet the antibiotic drug definition of "... inhibits in dilute solution ..." and, if so, should lovastatin and related drugs be considered for reclassification as antibiotic drug products under Section 507 of the Federal Food, Drug, and Cosmetic Act? The following data evaluation is intended to provide a reference framework for making that determination.

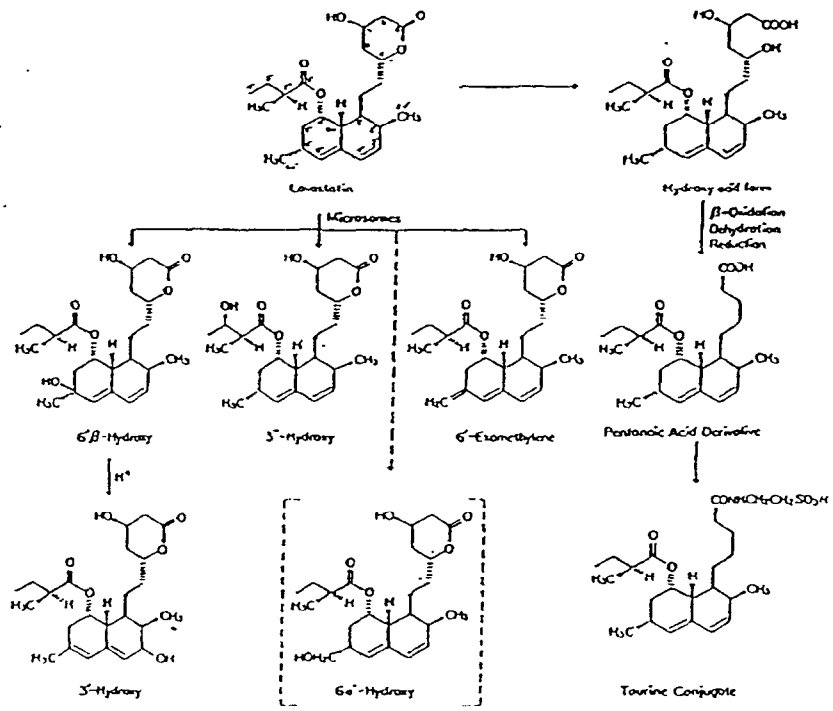
CHEMISTRY

Lovastatin and simvastatin are inactive lactone prodrugs, which after oral ingestion, are hydrolyzed to their corresponding, biologically active, beta-hydroxy acid forms. Pravastatin is marketed as the active beta-hydroxy acid form. Biotransformation of these drug products to several active and inactive metabolites has been reported (Vyas, et al , 1990a; Vyas, et al., 1990b; Halpin, et al., 1993) (Fig. 1). The 6a'-hydroxy-epi-lovastatin, an *in vivo* metabolite found in human and dog plasma, was not detected as a metabolite of rat or mouse liver microsomes. The inactive pentanoic acid derivative, a major metabolite resulting from beta-oxidation of the hydroxy acid form of lovastatin, has been detected in mice and rats; however, it has not been identified as a metabolite in humans.

Lovastatin, simvastatin and pravastatin are competitive inhibitors of HMG-CoA reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, which is an early and rate-limiting step in the biosynthesis of isoprenoid compounds that are intermediates in multiple biosynthetic pathways for biological molecules, including cholesterol, associated with numerous critical organism functions (Brown, et al , 1980) (Fig 2)

Figure 1.

Biotransformation pathway of lovastatin
(Vyas et al. 1990)



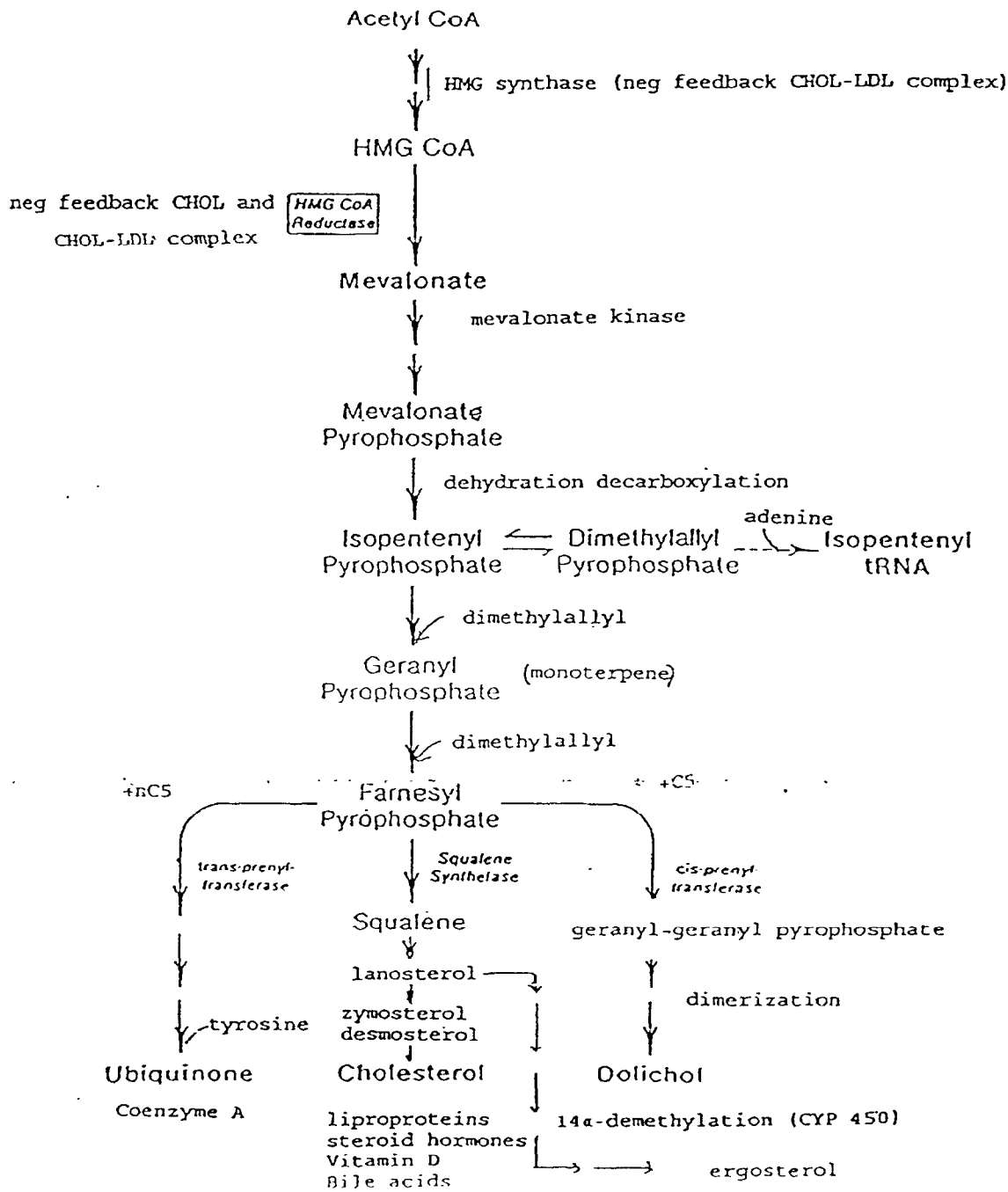
Enzyme-inhibitory activity of lovastatin and its metabolites

Compound	Relative activity*	
	Before hydrolysis	After hydrolysis
Lovastatin	0	100
Hydroxy acid form	100	100
6'-β-Hydroxy	0	60
3''-Hydroxy	0	15
6'-Exomethylene	0	50
3'-Hydroxy	0	0
Taurine conjugate	0	0
Pentanoic acid derivative	0	0

* Inhibitory activity of metabolites is expressed relative to the hydroxy acid form of lovastatin.

Figure 2.

Branched Pathway of Mevalonate Metabolism
Modification of Brown et al. 1980



PHARMACOLOGY

Human pharmacokinetics of HMG-CoA reductase inhibitors in plasma have been reviewed by Desager, et al., 1996. Tables I and II from their publication, showing data from multiple published studies, are reproduced below. These data are relevant in that they provide some insight into the drug concentrations to be considered when determining if the definition of "... inhibits in dilute solution ..." has been met. For lovastatin, the recommended dosing range for the treatment of hypercholesteremia is 10-80 mg/day in single or two divided doses; the maximum recommended dose is 80 mg/day. For simvastatin, the recommended dosing range is 5-40 mg/day as a single dose in the evening; the maximum recommended dose is 40 mg/day.

Table I. Main pharmacokinetic parameters of lovastatin (mean ± standard deviation)

Therapeutic daily dose (mg)	C_{max} (ugEq/L)		T_{max} (hours)		AUC (ugEq/L·h)[0-24h]		CL/F (L/h)*	
	AI	TI	AI	TI	AI	TI	AI	TI
80 (17 days)	40.7 ± 5.9	49.6 ± 8.3	2.0 ± 0.9	3.1 ± 2.9	305.2 ± 115.7	385.3 ± 107.3	262.1	207.6
80 (single dose-^{14}C)	70.7 ± 61.2	150.6 ± 106.9	2.3 ± 1.3	1.9 ± 1.2	282.4 ± 138.3	570.2 ± 275.4	283.3	140.3
40 (5 days)	45.5 ± 31.5	85.1 ± 58.2	2.41 ± 1	1.8 ± 1.4	236.0 ± 132.3[b]	359.1 ± 205.1	169.5	111.4
40 (7 days)	33.0 ± 9.8	65.7 ± 30.0	2.6 ± 1.3	2.3 ± 1.3	176.9 ± 72.4	284.6 ± 110.5	226.1	140.5
40 (single dose)	9.5 ± 5.2	19.9 ± 8.0	2.9 ± 1.7	2.6 ± 1.7	61.1 ± 72.0	114.1 ± 87	654.7	350.6
20 (single dose)	14.5 ± 8.8	27.1 ± 15.6	2.4 ± 1.2	2.1 ± 1.2	76.3 ± 41.6	114.2 ± 57.6	262.1	175.1

*Calculated from mean values.
[b]0 to 12 hours
Abbreviations: AI = active inhibitors; AUC = area under the plasma concentration-time curve from zero to 24 hours; CL/F = apparent total body clearance; ugEq = ug equivalent; TI = total inhibitors; T_{max} = time to reach peak concentration after drug administration.

Table II. Main pharmacokinetic parameters of simvastatin (mean ± standard deviation)

Therapeutic daily dose (mg)	C_{max} (ugEq/L)		T_{max} (hours)		AUC (ugEq/L·h)[0-24h]		CL/F (L/h)*	
	AI	TI	AI	TI	AI	TI	AI	TI
40 (17 days)	45.8 ± 19.5	56.5 ± 24.7	1.4 ± 1.0	1.4 ± 1.0	130.0 ± 32.0	172.0 ± 49.0	307.7	232.5
40 (single dose)	10.3 ± 6.9	34.5 ± 17.3	2.5 ± 1.7	2.3 ± 1.4	40.8 ± 26.3	102.5 ± 45.0	980.4	390.2
20 (single dose)		18.4 ± 7.3		1.7 ± 1.0		61.9 ± 20.6		323.1
100 (single dose - ^{14}C)[b]		125.0 ± 80.0		3.0		1020		98.0
20 (single dose)	9.9 ± 3.4		2.1 ± 1.3		39.6 ± 26.2		505	

*Calculated from the mean values.
[b]Patient with T-tube drainage.
Abbreviations: see table I above.

Other parameters of interest include protein binding effects, adsorption, total body tissue distribution, excretion, and half-life of lovastatin and simvastatin. In plasma, the hydroxy acid and lactone forms of lovastatin are 96 and 98.5% protein bound, respectively. For simvastatin, protein binding for these forms is 98 and 94.5%, respectively. Adsorption for lovastatin and simvastatin is approximately 31% and 60%, respectively. After absorption, these drugs undergo extensive first pass extraction by the liver, their primary site of action. The hydroxy acid form is less efficiently extracted

by the liver than the lactone. The metabolism of lovastatin and simvastatin by the liver is a permanent dynamic process because of the reversibility of the lactone to beta-hydroxy acid reaction. Thus, at any given time, lovastatin will be represented in tissue both as an active hydroxy acid form and as an inactive lactone form. For this reason, publications showing pharmacokinetics data often report "statin" drug concentrations measured as ugEquivalents/ml plasma rather than as ug/ml.

The excretion of inactive metabolites of lovastatin and simvastatin is mainly in feces (64% to 83%) and in urine (10% to 20%). The plasma $t_{1/2\beta}$ ranges from 3 to 4 hours. The pharmacokinetic half-lives are substantially less than the pharmacodynamic values, which are around 20 hours. The apparent total body clearance (CL/F) is very high due to the important first-pass liver extraction effect. Information concerning drug concentrations in other human tissues is limited in the literature and much of our information concerning tissue concentrations of drug are derived from animal studies. Duggan, et al., 1989, have evaluated lovastatin concentrations in numerous tissues of the rat and dog as shown in the table below.

Table 4 from Duggan et al., 1989.

Tissue distribution of lovastatin equivalents in rats and dogs

All values are ug equivalents per g (ml) of tissue. for rats, N = 3; for dogs, N = 4.

Tissue	Rat						Dog, po (60 mg/kg)
	iv (0.8mg/kg)			po (8 mg/kg)			
	1 hr	4 hr	24 hr	1 hr	4 hr	24 hr	4 hr
Plasma	0.23 ± 0.01	0.07 ± 0.01	0.02 ± 0.01	0.28 ± 0.04	0.27 ± 0.05	0.1 ± 0.01	0.27 ± 0.1
Heart	0.21 ± 0.05	0.03 ± 0.01	<0.02	0.48	0.2	<0.2	0.27 ± <0.1
Lung	0.23 ± 0.04	0.07 ± 0.02	<0.02	<0.5	<0.2	<0.2	0.41 ± 0.2
Liver	2.62 ± 0.83	0.62 ± 0.1	0.15 ± 0.06	6.57 ± 1.13	2.83 ± 0.9	1.01 ± 0.18	4.36 ± 2.0
Spleen	0.08 ± 0.02	0.04 ± 0.01	<0.02	<0.1	0.18 ± 0.06	<0.2	NS*
Adrenal	<0.28	<0.19	NS	<0.4	0.60	0.56 ± 0.05	0.22 ± 0.12
Kidney	0.39 ± 0.07	0.13 ± 0.02	0.04 ± 0.02	0.58 ± 0.04	0.47 ± 0.09	0.2 ± 0.03	0.71 ± 0.26
Stomach	0.12 ± 0.02	0.05 ± 0.01	0.02 ± 0.01	7.59 ± 5.02	12.16 ± 4.42	0.3 ± 0.08	NS
Small Intestine	2.52 ± 0.19	0.54 ± 0.08	0.07 ± 0.02	17.25 ± 5.51	11.26 ± 5.42	0.49 ± 0.13	13.62 ± 9.35
Large Intestine	0.14 ± 0.01	0.84 ± 0.23	0.05 ± 0.01	0.02 ± 0.07	5.2 ± 1.07	0.65 ± 0.48	NS
Testes	0.16 ± 0.01	0.03 ± 0.01	<0.02	<0.1	0.09 ± 0.01	0.04 ± 0.01	0.21 ± 0.02
Muscle	0.09 ± 0.03	<0.02	<0.02	<0.1	0.12	<0.1	0.35 ± 0.15
Fat	0.11 ± 0.02	0.02 ± 0	0.02	<0.1	0.12	0.29	NS
Brain	0.06 ± 0	0.02 ± 0	<0.03	0.1	0.08 ± 0.02	0.05 ± 0.01	0.17 ± <0.1

*NS, not significant.

The data from the above tables (Tables 1 and 2) would suggest that a maximum approved human dose of either lovastatin or simvastatin, administered chronically on a daily basis, would be expected to result in a C_{max} plasma drug concentration of approximately 40-60 ugEq/L (~0.1 uM) at steady state conditions. With a t_{max} occurring at 2-3 h and a $t_{1/2\beta}$ of 3 to 4 h, the trough plasma drug concentrations evident within 8 to 11 h would be expected to be ≤ 10-15 ugEq/L (~0.025 uM) following once daily oral dosing. Plasma drug concentrations following a single administered dose were slightly higher, but a single dose of drug would not be expected to provide activity of sufficient duration to treat an infectious disease. Interpretation of the data provided for tissue concentrations in rats and dogs treated with lovastatin suggests that with the exception of the liver, stomach, and intestines other body tissues exhibit lovastatin concentrations similar to or less than that observed in plasma. Although the human equivalent dose is different from that administered to these animals, lovastatin in humans is expected to exhibit a similar tissue distribution profile, relative to plasma concentrations, as that shown above for the rat and dog. Therefore, with respect to human clinical use of lovastatin and simvastatin, the target definition for "... inhibits in dilute solution ..." relevant to preclinical studies of antimicrobial activity drug concentrations should lie somewhere between 10 and 60 ugEq/L (~0.025 to 0.1 uM). The lovastatin concentration of 10 ugEq/L would represent that expected during the trough concentration phase while the 60 ugEq/L concentration would represent the upper range of C_{max} reported. However, because the pharmacokinetic half-lives are substantially less than the pharmacodynamic values of approximately 20 h, the trough concentrations may not be relevant to antimicrobial activity evaluation. Consequently, the C_{max} concentration of ~0.1 uM may represent a better choice for relevant comparisons to be made.

MICROBIOLOGY

Publications containing data relevant to possible antimicrobial activity of lovastatin and related "statin" drugs are presented below. The publications were evaluated and summarized independently to ensure that potentially critical parameters pertaining to each study were not co-mingled. A complete citation for each reference summarized is provided in bold type. Activity data are grouped by microorganism classification for ease of reference. A general summary of the data is provided in Tables 1-4 in the discussion section at the end of this report.

Lovastatin Antimicrobial Activity Against Bacteria:

Zhou, D., et al. Early steps of isoprenoid biosynthesis in Escherichia coli. Biochem J. 1991 Feb 1; 273(Pt 3): 627-34.

In this paper the authors reported the lack of involvement of mevalonic acid in the early steps of isoprenoid biosynthesis in *E. coli*. Mevinolin (lovastatin) at concentrations as high as 68.3 uM did not affect growth of *E. coli*. Interpretation of data presented in this paper (while not ruling out involvement of non-membrane bound mevalonate) would suggest that eubacteria, such as *E. coli*, do not utilize acetyl-CoA and mevalonic acid in the biosynthesis of isoprenoids as has been reported for archaebacteria and eukaryotes (*vide infra*). The authors suggested that if the alternative pathway for biosynthesis of isoprenoids in *E. coli* is a general characteristic of all eubacteria, then it may represent a clear biochemical marker that separates eubacteria from archaebacteria and eukaryotes. If true, inhibitors of HMG-CoA reductase, such as lovastatin, would not be expected to inhibit growth of species of true bacteria. Data showing lovastatin growth inhibition of any bacteria other than those classified as archaebacteria were not found in the published literature.

Cabrera, JA., et al. Isoprenoid synthesis in Halobacterium holobium. Modulation of 3-hydroxy-3-methylglutaryl coenzyme A concentration in response to mevalonate availability. J Biol Chem. 1986 Mar 15; 261(8): 3578-83.

In this paper, the authors utilized *H. holobium*, a genus of organism representative of those archaebacteria which require >15% NaCl for growth [Dundas, I.E.D. (1977) Adv. Microb. Physiol. 15, 85-120; cited in Cabrera, et al., 1986], as a unique biological model to study the regulation of mevalonate synthesis. They reported data which supports the conclusion that *H. holobium*'s HMG-CoA concentration, and not HMG-CoA reductase activity, was reversibly modulated in response to mevalonate availability, in contrast that reported for eukaryotic cells As part of their experimental design, they evaluated mevinolin (lovastatin) induced effects on mevalonate content of *H. holobium*. They demonstrated that growth of *H. holobium* was completely inhibited by mevinolin (lovastatin) at concentrations of 1-2 uM (~0.4 to 0 8 ug/ml) This inhibition by lovastatin was reversed by the addition of 4 mM mevalonate to the culture medium.

Lam, WL., et al. Shuttle vectors for the archaebacterium Halobacterium volcanii. Proc Natl Acad Sci USA. 1989 Jul; 86(14): 5478-82.

In this publication, the authors reported that lovastatin completely inhibited *in vitro* growth of the *Archaebacterium*, *H. volcanii* strain WFD11, at 1-2 uM (~0.4 to 0.8 ug/ml) and at 20-40 uM (~8 to 16 ug/ml) when cells were grown on agar prepared with minimal or enriched medium, respectively. The differential sensitivity of microorganisms to lovastatin inhibition when grown on medium with and without lipids is a commonly reported observation in the published literature.

Lovastatin Antimicrobial Activity Against Viruses:

Overmeyer, JH. Isoprenoid requirement for intracellular transport and processing of murine leukemia virus envelope protein. J Biol Chem. 1992 Nov 5; 267(31): 22686-92.

In this publication, the authors examined the potential relationship between isoprenoid biosynthesis and the processing of murine leukemia virus (MuLV) envelope glycoprotein in murine erythroleukemia (MEL) cells cultured in Dulbecco's Minimum Essential Medium (MEM) with 10% fetal bovine serum. They reported that lovastatin, at concentrations as low as 1 ug/ml (~2.5 uM), was not cytostatic for MEL cells in culture, but prevented the cells' ability to convert MuLV envelope glycoprotein precursor, $gPr90^{env}$, to the mature envelope glycoprotein, $gp70^{env}$. This conversion normally occurs within the Golgi apparatus. It was suggested that lovastatin may prevent viral envelope precursors from reaching the Golgi compartment by blocking the geranylgeranyl isoprenylation of the GTP-binding *rab* proteins required for the transport of precursor viral glycoprotein from the endoplasmic reticulum (ER) to the Golgi apparatus. In cells infected with retroviruses, the envelope glycoproteins encoded by the viral *env* genes normally undergo proteolytic processing and oligosaccharide maturation upon translocation from the ER to the Golgi apparatus. Inhibition of proteolytic cleavage of viral envelope proteins is known to reduce infectious virus titers. The authors reported that the lovastatin inhibitory effect on envelope maturation was drug dose dependent and was completely reversed by the addition of 200 uM mevalonate to the culture medium. However, the authors did not report the effect, if any, of lovastatin on MuLV infectivity.

Maziere, JC., et al. Lovastatin inhibits HIV-1 expression in H9 human T lymphocytes cultured in cholesterol-poor medium. Biomed Pharmacother. 1994; 48(2): 63-7.

In this publication, the authors investigated the *in vitro* effect of lovastatin on HIV production in H9 T lymphocytes adapted to grow in RPMI 1640 medium supplemented with only 1% human serum to limit exogenous cholesterol supply. Lovastatin (0.3 uM final concentration) (~0.12 ug/ml) was added to the culture medium 1 day post-infection. The medium was replaced each day by new medium containing the same concentration of lovastatin. Reverse transcriptase activity was reduced approximately 10-fold after 9 days of lovastatin treatment compared to untreated, infected controls. The authors concluded from these data that clinical intervention that would lower cholesterol availability for HIV viral membrane synthesis may have some benefit in treatment of viral replication in human AIDS patients. The effects of adding additional exogenous cholesterol or serum on the observed antiviral activity was not investigated.

Malvoisin, E., et al. Effect of drugs which inhibit cholesterol synthesis on syncytia formation in vero cells infected with measles virus. Biochim Biophys Acta. 1990 Feb 23; 1042(3): 359-64.

For these studies, Vero cells were infected with measles virus (Hallé strain) and incubated in Eagle's minimum essential medium containing 2% fetal calf serum and antibiotics (100 units/ml penicillin and 100 ug/ml streptomycin). Inhibitors of cholesterol biosynthesis [including mevinolin at 6 ug/ml (~15 uM)] inhibited measles virus induced syncytia in Vero cells, but this effect was not necessarily related to an inhibition of virus infectivity. Inhibition of virus infection occurred with some non-statin cholesterol synthesis inhibitors, but appeared to be due to the inhibitor's effects on parameters other than cholesterol synthesis. Inhibition of virus infection by mevinolin was not reported. Furthermore, cell cytotoxicity related to mevinolin was not reported. Thus, although mevinolin significantly reduced syncytia formation in measles virus infected Vero cells, antiviral activity was not reported to be associated with this effect.

Lovastatin Antimicrobial Activity Against Yeast and Fungi:

Ikeura, R., et al. Growth inhibition of yeast by compactin (ML-236B) analogues. J Antibiot Tokyo. 1988 Aug; 41(8): 1148-50.

In this publication, a variety of HMG-CoA reductase inhibitors, including lovastatin (monacolin K), were evaluated for antimicrobial activity against 303 strains of yeast representing 41 genera and 165 species. All of the HMG-CoA reductase inhibitors were converted to their respective active hydroxy acid form by hydrolysis prior to use.

Yeast strains were inoculated onto 0.67% yeast nitrogen base medium containing 0.5% glucose and 1.5% agar (pH 5.3), and grown at 30 C. Where indicated, compactin (lovastatin is an analogue of compactin) was supplemented to the medium at a concentration of 0-20 ug/ml (~0-50 uM). Growth was inspected after 4 days of cultivation. The authors stated that of 303 strains tested, 43 strains (18 genera, 35 species), 21 strains (13 genera, 19 species) and 4 strains gave no detectable growth on the agar medium containing 20, 10 and 4 ug/ml of compactin, respectively (50, 16, and 10 uM, respectively). The remaining 260 strains (34 genera, 135 species) were resistant to compactin at 20 ug/ml (~50 uM), data not shown. The most sensitive 4 strains were *Rhodotorula glutinis* H3-9-1, *Sporobolomyces salmonicolor* WF 188, *Aessosporon salmonicolor* IFO 1845 and *Citeromyces matritensis* IFO 0954 with MIC values of 0.1, 1.0, 2.0 and 2.0 ug/ml, respectively (range ~0.25 to 5 uM). The identity of the remaining 299 strains was not reported.

Growth inhibition was subsequently determined for *R. glutinis* H3-9-1 and *S. salmonicolor* WF 188 in liquid medium consisting of 0.67% yeast nitrogen base and 0.5% glucose. Inhibitors were added at concentrations of 0-100 ug/ml (~0 to 250 uM) and cells were cultured with shaking at 30 C for 4 days. Growth was monitored by measuring OD at 550 nm. Monacolin K (lovastatin) and compactin were the most potent inhibitors having MIC values of 0.1 and 1.0 ug/ml (~0.25 and ~2.5 uM) for *R. glutinis* H3-9-1 and *S. salmonicolor* WF 188, respectively. Inhibitory activity of the other HMG-CoA reductase inhibitors (ML-236A, monacolin L, and monacolin X) were 1/25 - 1/50 of the above values.

In a separate experiment, the ability of mevalonate to reverse the inhibition of compactin against the 4 most sensitive strains mentioned above was evaluated in a dose dependent study. At 10 mM, mevalonate completely reversed the compactin inhibition for all strains except for *Citeromyces matritensis* IFO 0954. However, the growth curve for *C. matritensis* IFO 0954 in the absence of compactin was substantially reduced when compared to the growth curves of the other strains grown under the same conditions. This observation suggests that under normal culture conditions, growth of *C. matritensis* IFO 0954 was aberrant and compactin inhibition was substantially more detrimental under these circumstances. Thus, the relevance of the inhibition pattern for *C. matritensis* is difficult to interpret.

Lorenz, RT., et al. Effects of lovastatin (mevinolin) on sterol levels and on activity of azoles in Saccharomyces cerevisiae. Antimicrob Agents Chemother. 1990 Sep; 34(9): 1660-5.

In this publication, the authors reported the quantitative effects of lovastatin on the free sterol and steryl ester fractions of wild type *Saccharomyces cerevisiae*, strain 2180-1A. In these studies, the organisms were grown in medium (YPD) consisting of 2% glucose, 1% peptone, and 1% yeast extract. Minimal inhibitory concentrations (MICs) were determined by inoculating 5 ul of an overnight culture into YPD medium and incubating at 28° C with constant shaking. The MICs were recorded as the lowest concentration of antifungal agent at which no significant visible growth occurred after 3 days. Lovastatin lactone prodrug that was used in this study was hydrolyzed to the active hydroxy acid form prior to use.

Lovastatin at 10 ug/ml (~25 uM) was reported to dramatically decrease the total endogenous steryl ester fraction in *S. cerevisiae*. As the concentration of lovastatin increased progressively above 10 ug/ml, the free sterol fraction decreased linearly. Moreover, in addition to severely decreasing the accumulation of endogenous steryl esters , lovastatin prevented the esterification of sterol taken up from the medium. However, the growth rate and cell yield were not significantly affected until a lovastatin concentration of 75 ug/ml (~190 uM) or greater was present in the medium; at concentrations above 150 ug/ml (~380 uM), the growth rate and cell yield were severely diminished (data not shown).

In combination studies, *S. cerevisiae* was grown with different amounts of lovastatin and ketoconazole, clotrimazole or miconazole. Interpretation of the results obtained indicated that there was a synergistic effect of lovastatin and different azoles in lowering the MICs of azole antifungal agents. Lovastatin at 2 ug/ml (~5 uM)significantly decreased the MICs of each azole. In the presence of lovastatin at 10 ug/ml (~25 uM), the MICs of clotrimazole, ketoconazole, and miconazole were decreased 6-, 10-, and 32-fold, respectively. The authors hypothesized that the synergism observed between lovastatin and these azoles may be due to increased cell membrane permeability caused by the effect of lovastatin on the sterol content of the organism. The authors reported that *S. cerevisiae* cell membrane permeability to

exogenous sterols was increased under conditions where endogenous sterols were decreased (see above). They speculated that as membrane permeability was increased for sterols then it may be increased for other agents, such as azoles, as well. However, data were not provided to demonstrate that intracellular concentrations of azoles occurred under these conditions.

Sud, IJ., et al. Effect of ketoconazole in combination with other inhibitors of sterol synthesis on fungal growth. Antimicrob Agents Chemother. 1985. 28: 532-534.

The authors of this publication evaluated, *in vitro*, the inhibitory effects of ketoconazole, mevinolin (lovastatin) and a combination of these two drugs against a variety of fungi. The data below are taken from Tables 1 and 2 of their publication.

Sterol synthesis inhibitors

Fungus tested	MIC (ug/ml) of inhibitor		Concn (ug/ml) of inhibitor giving a fourfold or greater decrease in the MICs of ketoconazole
	Ketoconazole	Mevinolin	Mevinolin
Candida albicans VA	0.045	50	3.12 (4)[a]
Candida albicans 7.22	3.12	100	25.0 (8)
Candida tropicalis	0.78	>100	--[b]
Torulopsis glabrata	0.78	>100	--
Aspergillus fumigatus 173	3.12	6.25	3.12 (8)
Aspergillus fumigatus	6.25	6.25	0.78 (4)
Aspergillus niger	12.5	12.5	0.78 (4)[c]
			1.56 (8)
Rhizopus rhizopodiformis	6.25	50	12.5 (4)
			25.0 (8)

[a]Numbers in parentheses represent the fold decrease in the MIC of ketoconazole in the presence of the indicated concentrations of Mevinolin.
[b]No change or less than a fourfold decrease in the MIC of ketoconazole in the presence of mevinolin.
[c]Where more than one number is given, the lower number is the concentration of the drug giving a fourfold decrease in the MIC of ketoconazole, and the higher number is the concentration showing the maximum effect.

These data were generated in *in vitro* studies utilizing completely synthetic media. The species most sensitive to mevinolin (lovastatin) were *A. fumigatus* and *A. niger* with MICs of 6.25 and 12.5 ug/ml (~16 uM and ~32 uM), respectively. These species were also the ones showing the most sensitivity to the combination effects (4- to 8-fold decrease in MICs of ketoconazole) of ketoconazole and lovastatin. The ability of intermediates of the isoprenoid and steroid pathways, subsequent to mevalonic acid synthesis, to reverse the inhibitory effects of lovastatin observed in this study was not evaluated.

Bejarano, ER., et al. Independence of the carotene and sterol pathways of Phycomyces. FEBS Lett. 1992 Jul 20; 306(2-3): 209-12.

In this publication, the authors evaluated the pathway for the synthesis of carotene and sterols in *Phycomyces blakesleeanus* and various mutants with altered carotenogenesis. The fungus was grown on minimal agar medium at 22° C in the dark. Lovastatin and mevalonic acid lactone were hydrolyzed to the hydroxy acid forms prior to addition to growth medium. *Phycomyces* did not grow on medium with 1 uM (~0.4 ug/ml) lovastatin. This inhibition was reversed by the presence of mevalonate in the medium at 10 mM, but not at 1 mM.

Engstrom, W., et al. The effects of tunicamycin, mevinolin and mevalonic acid on HMG-CoA reductase activity and nuclear division in the myxomycete Physarum polycephalum. J Cell Sci. 1989 Mar; 92(Pt 3): 341-4.

In this publication, the authors reported that lovastatin at concentrations ≥ 25 uM (~10 ug/ml), inhibited protein synthesis, DNA synthesis, nuclear division and plasmodia growth, *in vitro*, of *Physarum polycephalum*. These effects could be partially reversed by the addition of mevalonate at concentrations ≥ 0.4 mM.

Lovastatin Antimicrobial Activity Against Parasites:

Andersson, M., et al. Lovastatin inhibits interferon-gamma-induced Trypanosoma brucei proliferation: evidence for mevalonate pathway involvement. J Interferon Cytokine Res. 1996 Jun; 16(6): 435-9.

In this publication, the authors reported that interferon-gamma, at low concentrations (10^3 U/ml added to 10^6 parasites), had a growth stimulatory effect on *Trypanosoma brucei brucei in vitro* and that this proliferative response was blocked by low levels of lovastatin (0.1 uM) (~.04 ug/ml). However, lovastatin did not inhibit growth at concentrations as high as 20 uM (~8 ug/ml), the highest concentration tested, when added to nonstimulated cultures of the parasite.

> **Note:** In this study, lovastatin concentration was given as uM in the figures, but was given as mM in the figure legends and in the text of the paper. Lovastatin is insoluble in water (Mevacor package insert). Therefore, it is assumed, but not known with certainty, that the values listed as uM were the correct concentrations to use in this report.

Florin-Christensen, M., et al. Inhibition of Trypanosoma cruzi growth and sterol biosynthesis by lovastatin. Biochem Biophys Res Commun. 1990 Feb 14; 166(3): 1441-5.

In this publication, the authors report a dose dependent lovastatin inhibition of the *in vitro* growth of *Trypanosoma cruzi* epimastigotes at 10 and 30 ug/ml (~25 and ~75 uM, respectively). Squalene at 100 uM, but not cholesterol, reversed lovastatin's growth inhibitory effects induced by 10 and 30 ug/ml suggesting that lovastatin interfered with steps leading to squalene biosynthesis. At 50 ug/ml (~125 uM), lovastatin killed most of the trypanosomes. Squalene was not able to reverse the inhibitory effects on epimastigotes treated with 50 ug/ml of lovastatin.

Haughan, PA., et al. Synergism in vitro of lovastatin and miconazole as anti-leishmanial agents. Biochem Pharmacol. 1992 Dec 1; 44(11): 2199-206.

In this publication, the authors reported on the *in vitro* combinational use of the antifungal drug, miconazole, with the cholesterol lowering drug, lovastatin, to assess their potency as anti-leishmanial agents. Activity was assessed for each drug as single agents and in combination against Leishmania promastigotes and amastigotes.

Lovastatin, as a single drug, had IC_{50} values of 82 ug/ml (~200 uM) and 20 ug/ml (~50 uM) against *L. donovani* and *L. amazonensis* promastigotes, respectively. Miconazole, as a single drug, had IC_{50} values of 6 and 3 ug/ml, respectively, against these life-cycle forms. Treatment of *L. amazonensis* amastigotes in mouse peritoneal macrophages with lovastatin up to a concentration of 10 ug/ml (~25 uM) had little effect on the percentage of macrophages infected or the number of amastigotes in the macrophages. Due to drug insolubility problems, and IC_{50} could not be determined, but it was estimated to be well in excess of 10 ug/ml (~25 uM). The IC_{50} for miconazole was estimated to be 8 ug/ml.

When used in combination, miconazole and lovastatin IC_{50} concentrations of each drug could be reduced by 2- to 10-fold, suggesting a synergistic activity interaction against these life-cycle forms of these *Leishmania spp.*

Morrison, DD., et al. Effects of steroids and steroid synthesis inhibitors on fecundity of S. mansoni in vitro. J Chem Ecol. 1986; 12: 1901-08.

Mevinolin (lovastatin) was reported to significantly depress egg production (~50%) at 1 uM (0.4 ug/ml) in *Schistosoma mansoni* grown *in vitro* for 72 h at 37°C with shaking in medium that was a 1:1 mixture of RPMI 1640 and heat-inactivated horse serum, adjusted to pH 7.4. Penicillin and streptomycin (100 ug/ml each) were added along with mercaptoethanol to a final concentration of 5×10^{-5} M. Mevinolin at higher concentrations (i.e., 10 uM and 100 uM) (~4 and ~40 ug/ml, respectively) was unable to completely inhibit egg production. Effects on adult mating pairs appeared to be minimal even at 100 uM lovastatin. Adult schistosomes are incapable of *de novo* cholesterol formation [(Meyer et al., 1970; Smith et al., 1970); cited by the authors of this paper]. Egg production inhibition by lovastatin was not reversed by coincubation with 100 uM cholesterol. Morrison, et al., concluded from these data that lovastatin inhibition of egg production is not due to a steroid-mediated effect.

Vandewaa, EA., et al. Physiological role of HMG-CoA reductase in regulating egg production by Schistosoma mansoni. Am J Physiol 1989 Sep; 257(3 Pt 2): R618-25.

The purpose of this publication was to provide evidence suggesting that HMG-CoA reductase activity plays a critical role in parasite egg production. Several lines of evidence, described below, were provided to support this hypothesis.

White outbred (ICR) female mice, infected intraperitoneally with 250-300 schistosome cercariae, were dosed daily with lovastatin (50 or 250 mg/kg) by gavage for 3 days starting at 42 days postinfection. Control mice were dosed with vehicle only. After treatment of these acutely infected mice, parasites were collected and microsomes were prepared. HMG-CoA reductase enzyme activity measured in microsomes obtained from schistosomes exposed to 250 mg/kg lovastatin was reduced significantly (~3-fold) compared to untreated controls. However, if the lovastatin exposed parasites were subsequently grown *in vitro* for 24 h in drug free medium prior to assay for HMG-CoA reductase activity, the enzyme activity was observed to be significantly enhanced (~2-fold) over controls. In contrast to these results, parasites collected from mice treated with 50 mg/kg lovastatin were shown to have a significant induction in HMG-CoA reductase activity over controls.

Because low doses of lovastatin (50 mg/kg vs. 250 mg/kg) produced higher levels of HMG-CoA reductase activity in the above experiments, egg production in schistosomes obtained from lovastatin treated mice, dosed daily for 10 days at 50 mg/kg starting at 35 days postinfection, was evaluated *in vitro* (Table 2 from Vandewaa, et al., 1989).

Table 2. Effect of mevinolin on *in vitro* egg production by *S. mansoni* after *in vivo* exposure to the drug

In Vivo Treatment	Concentration of Mevinolin in Culture Media	No. Eggs
Vehicle	0	60.4 ± 32.6
Vehicle	10 uM	10.7 ± 7.3*
Mevinolin (50 mg/kg)	0	321.8 ± 90.4*
Mevinolin (50 mg/kg)	10 uM	6.3 ± 4.1*

Data are means ± SD for number of eggs per female per 72 h. Parasites were incubated in the presence or absence of mevinolin following *in vivo* exposure to the drug or its vehicle. *Significantly different from control, $P < 0.01$.

These results show that adult *S. mansoni* schistosome egg production, measured in an *in vitro* assay, was stimulated approximately 5-fold in infected mice treated with 50 mg/kg lovastatin. Moreover, this stimulation could be blocked

upon the addition of 10 uM mevinolin to the *in vitro* culture medium. Furthermore, it was reported that lovastatin's *in vitro* inhibition of schistosome egg production could be reversed by the addition of either farnesol or mevalonate at a concentration of 80 uM. These data, taken together with the fact that schistosomes are incapable of synthesizing cholesterol *de novo*, led these authors to conclude that a nonsterol lipid, yet to be identified, may play an important role in regulating egg production by *S. mansoni*.

These *in vitro* observations led to experiments wherein *in vivo* egg production by schistosomes was measured in mice treated with 50 or 250 mg/kg lovastatin. Drug was administered by oral gavage for 10 days beginning 35 days postinfection. The results on *in vivo* egg production correlated with observations on HMG-CoA reductase enzyme activity and on *in vitro* egg production, mentioned above. At 50 mg/kg, egg production, *in vivo*, was enhanced over that observed in control animals (degree of enhancement not reported). In mice treated with 250 mg/kg, egg production was inhibited 45.4% compared to control animals. This reduction in egg production was correlated with a reduction in liver pathology associated with schistosome infections in mice. Reduction in pathology did not occur in infected mice treated with lovastatin at 50 or 100 mg/kg. Adult worm burden was unaffected by treatment with lovastatin at any of the concentrations evaluated.

From these studies, the authors concluded that "... Although the chronic application of mevinolin to an infected human would be an inappropriate strategy for the control of the disease associated with the infection, we felt that the consequences of a continuous application of mevinolin to infected mice should validate the concept that a reduction in egg production should reduce the parasite-induced pathology."

Chen, GZ., et al. Antischistosomal action of mevinolin: evidence that 3-hydroxy-methylglutaryl-coenzyme A reductase activity in Schistosoma mansoni is vital for parasite survival. Naunyn Schmiedebergs Arch Pharmacol. 1990 Oct; 342(4): 477-82.

This publication is an extension of the observations reported by Vandewaa, et al., 1989, described above. In this paper, these authors reported on adult and developing schistosome survival in mice administered 0.2% lovastatin (equivalent to 640 mg/kg/day) in the diet for 14 days, beginning 35 to 45 days postinfection. Results from this study show that 96-100% of adult parasites were eliminated by this treatment. These effects were shown to be drug dose dependent. Administration of the same dose beginning 2 days prior and continuing for 15 days after infection (juvenile stage of parasite growth), resulted in 93-96% reduction of adult parasites. To determine if lovastatin could be shown to be lethal in *in vitro* cultures of schistosomes, adult parasites were exposed to increasing doses of lovastatin (1 to 10 uM). Lactate production and motility in these treated parasites, as a measure of drug toxicity, were observed over time. The response was time and dose dependent. At 3 days incubation, 10 uM lovastatin reduced motility and lactate production > 50%, at 11 days of culture, doses of 1-10 uM inhibited activity nearly 90%. It was stated that inhibition of motility and lactate production eventually resulted in death of the organism but it was not clear from the results provided as to when death would actually occur.

Urbina, JA., et al. Mevinolin (lovastatin) potentiates the antiproliferative effects of ketoconazole and terbinafine against Trypanosoma (Schizotrypanum) cruzi: in vitro and in vivo studies. Antimicrob Agents Chemother. 1993 Mar; 37(3): 580-91.

In this study, the authors evaluated the potentiation effect of lovastatin on the antiproliferative effects of ketoconazole and terbinafine against *Trypanosoma cruzi*, the causative agent of American trypanosomiasis (Chagas' disease). Activity against epimastigotes and amastigotes *in vitro* and parasitemia *in vivo* was determined for each single drug and also for the drugs when used in combination. For all *in vitro* studies reported, lovastatin was hydrolyzed to the active hydroxy acid drug form prior to use.

In *in vitro* studies, the epimastigote form was cultivated in liver infusion-tryptose medium supplemented with 10% calf serum at 28° C with strong agitation (120 rpm). The antiproliferative effects were measured at various times after addition of varying concentrations of each drug alone and in combination. Results obtained from these *in vitro* studies showed that lovastatin, at 7.5 uM (3 ug/ml), ketoconazole at 0.1 uM and terbinafine at 1 uM, each, reduced growth of *T. cruzi* epimastigotes 20% to 30% when evaluated as single agents. Lovastatin at 50 and 75 uM (20 and 30 ug/ml, respectively) caused complete growth arrest with cell lysis ensuing at 144 and 96 h, respectively. Lovastatin at 7.5 uM in combination with ketoconazole at 0.1 uM resulted in complete growth arrest followed by cell lysis at 144 h. Thus, the authors concluded that the trypanocidal concentration of lovastatin was reduced by a factor of 10 in the presence of a ketoconazole concentration that by itself had only very modest effects on parasite growth. Terbinafine in combination with lovastatin produced a lesser effect, complete growth inhibition and lysis required 25 uM lovastatin with 1 uM terbinafine.

The authors also reported on the effects of lovastatin on *T. cruzi* amastigotes proliferating inside Vero cells *in vitro*. Lovastatin at 1 uM (0.4 ug/ml) produced less than a 30% reduction in the number of parasites per Vero cell and % of infected cells after incubation at 37° C for 96 h. At concentrations greater than 1 uM, lovastatin had a deleterious effect on the host cells; thus, the antiparasitic activity measured is close to the cytotoxic drug concentration for the Vero cells (i.e., the therapeutic index is close to 1). However, lovastatin at 0.75 uM in combination with 1 nM ketoconazole, which by itself produced a 30 to 40% reduction of in the number of infected cells, produced a complete elimination of amastigotes without deleterious effects on the host cells when cells were treated for 192 h. When terbinafine and lovastatin were evaluated in combination, only additive effects on amastigote reduction were observed. In these studies, amastigotes were cultivated in Vero cells maintained in minimal essential medium supplemented with 2% fetal calf serum in humidified 95% air-5% CO_2 atmosphere at 37° C. The medium, with and without drug, was changed every 48 h.

From their *in vivo* murine model of Chagas' disease, the authors reported the following results:

> "... mice treated orally with ketoconazole at 30 mg/kg of body weight per day for 7 days were fully protected from death 40 days after infection with a lethal inoculum of *T. cruzi* blood trypomastigotes, while all the controls (untreated) were dead 24 days postinoculation; ketoconazole at this dose completely suppressed parasitemia. When the dose of ketoconazole was lowered to 15 mg/kg/day, incomplete protection against death and significant numbers of circulating parasites were observed for up to 25 days. Mevinolin at 20 mg/kg/day promoted 50% survival, but the level of parasitemia was comparable to that observed in the controls. However, when the low dose of ketoconazole was combined with mevinolin, 100% survival and almost complete suppression of parasitemia were observed, indicating a synergic action in vivo, which was most evident in the effect on circulating parasites .. "

In these studies, drugs, suspended in 2% methylcellulose containing 0.5% Tween 80, were given by gavage once daily for 7 days. *T. cruzi* Y strain was inoculated (10^5 trypomastigotes) intraperitoneally into female outbred NMRI albino female mice weighing 25 to 30 g and treatment was initiated 24 h later.

Brener, Z., et al. An experimental and clinical assay with ketoconazole in the treatment of Chagas disease. Mem Inst Oswaldo Cruz. 1993 Jan-Mar; 88(1): 149-53.

In this publication the authors tested the *in vivo* activity of ketoconazole associated with lovastatin for possible synergistic activity against *T. cruzi* Y strain infection in mice (see table below). Other drugs evaluated in this publication were not reviewed for this report.

In this study, groups of Swiss albino mice, weighing 18-20 g were inoculated intraperitoneally with 2 x 10^4 blood forms of *T. cruzi* Y strain. Treatment was started 24 h after infection and continued for 20 days. Drugs were prepared in distilled water and administered by oral gavage.

Table II from Brener, et al., 1993.

Parasitemia and mortality in groups of mice inoculated with 2 X 10^4 blood forms of the *Trypanosoma cruzi* Y strain and treated with ketoconazole, lovastatin and association of both drugs

Drug (mg/kg)	No. parasites/5 ul (7^{th} day)	Mortality (20^{th} day)
Ketoconazole (100)	0	0/6
Ketoconazole (40)	780	1/6
Ketoconazole (25)	14.670	1/5
Lovastatin (100)	64.333	6/6
Ketoconazole (40) Lovastatin (10)	689	1/5
Ketoconazole (25) Lovastatin (25)	15.480	4/5
Untreated controls	19.021	5/5

Note: The data reported in this table is in conflict with statements made by the authors in the text. The numbers reported in the table for the No. parasites/5 ul for Ketoconazole (40) and the combination of Ketoconazole (40) with Lovastatin (10) appear to be incorrect based upon text information provided. Rather than the numbers 780 and 689 as reported above, the actual numbers may be 0.780 and 0.689, respectively. It appears that a decimal proceeding the number was omitted when printed by the publisher. It is not certain that this assumption is valid. However, the analysis of the results has been based upon the written text information which implies that this assumption is reasonable.

Interpretation of data presented in this publication shows that lovastatin at the highest dose evaluated (100 mg/kg) exacerbated parasitemia approximately 3-fold over untreated controls and failed to provide a survival benefit associated with treatment. Ketoconazole at 100 mg/kg eliminated the parasitemia and 100% of the mice survived. Ketoconazole at 25 mg/kg reduced parasitemia approximately 20% and 80% of the mice survived; whereas, all of the untreated controls died. If the assumption that the correct numbers for parasitemia in the groups of mice treated with ketoconazole, as discussed above, are 0.780 and 0.689, then the parasitemia data reported in this table suggest that lovastatin in combination with ketoconazole is antagonistic in this infection model with respect to parasitemia.

Lujan, HD., et al. Isoprenylation of proteins in the protozoan Giardia lamblia. Mol Biochem Parasitol. 1995 Jun; 72(1-2): 121-7.

The authors of this publication reported that *Giardia lamblia* has the ability to modify several of its cellular proteins by isoprenylation. Protein isoprenylation and cell growth were inhibited in a dose dependent manner with complete inhibition obtained by concentrations of compactin ≥200 uM (~80 ug/ml) and mevinolin (data were shown for compactin only). This inhibition due to HMG-CoA reductase inhibitors was completely reversible by the addition of 2 mM mevalonate to the culture medium.

Lovastatin and Simvastatin Antimicrobial Activity Against Parasites:

Grellier, P., et al. 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors lovastatin and simvastatin inhibit in vitro development of Plasmodium falciparum and Babesia divergens in human erythrocytes. Antimicrob Agents Chemother. 1994 May; 38(5): 1144-8.

In this publication, the authors evaluated the ability of lovastatin and simvastatin to inhibit, *in vitro*, growth and development of *Plasmodium falciparum* and *Babesia divergens*, the causative agents of human malaria and bovine babesiosis, respectively. *B. divergens*, in some cases, causes disease in humans.

Asynchronous parasite cultures (0.5% parasitemia and 1% hematocrit) of *P. falciparum* were maintained on human type O^+ RBC in RPMI 1640 culture medium supplemented with 27.5 mM $NaHCO_3$, 25 mM HEPES buffer (pH 7.4), 11 mM glucose, and 10% human O^+ serum in an atmosphere of 3% CO_2, 6% O_2, and 91% N_2 at 37° C. The *B. divergens* isolates were maintained *in vitro* in the same manner as *P. falciparum* except the cultures contained 1% parasitemia rather than 0.5%. Cultures were treated with lovastatin or simvastatin at various concentrations for 24 h. Parasite growth was estimated in lovastatin or simvastatin treated cultures either by [3H]hypoxanthine incorporation for 18 and 16 h, respectively, or by Giemsa-stained smears made at the end of the experiment. Results are shown in the following table.

Table 1 from Grellier, et al., 1994

Antiparasitic activities of HMG-CoA reductase inhibitors

	Mean IC_{50} ($ugml^{-1}$) ± SD	
	Lovastatin	Simvastatin
Parasite strain		
P. falciparum		
F32/Tanzania	15.7 ± 6.5[a]	16.2 ± 3.6[a]
FcB.1/Columbia	13.6 ± 3.7[b]	12.8 ± 2.5[b]
B. divergens		
Rouen 1987	8.4 ± 0.3[b]	5.0 ± 0.4[b]
Weybridge 8843	ND[c]	5.8[d]

[a] From four experiments.
[b] From three experiments.
[c] ND, not determined.
[d] From two experiments.

Similar IC_{50} values were obtained for lovastatin and simvastatin against the plasmodium strains; both IC_{50} values were in the range of 10 to 20 ug/ml (~25 to 50 uM). The drugs were equally effective against the chloroquine-susceptible F32/Tanzania stain and the chloroquine-resistant FcB.1/Columbia strain. IC_{50} values for *B. divergens* isolates were in the range of 5 to 10 ug/ml (~12.5 to 25 uM) and suggest no difference in sensitivity between the two strains tested.

Subsequent inhibition assays with 6-h-pulse incubations of simvastatin with *P. falciparum* synchronized cultures showed that the trophozoite stage of the erythrocytic life cycle is the stage at which the parasite is most susceptible to simvastatin. Cytotoxic effects giving a complete inhibition of growth were observed for all parasite stages only with drug concentrations above 50 ug/ml (~125 uM). Reversal of parasite growth inhibition by excess of exogenous mevalonate was unsuccessful and may have been due to the inability of non-drug treated *P. falciprum* infected RBC to incorporate [14C]mevalonate. This observation suggests that the parasite is not capable of mevalonate uptake from the assay medium

From these studies, the authors concluded that the achievable concentrations of HMG-CoA reductase inhibitors in human plasma are unsuitable for a blood eradication of malaria by the current usage of this cholesterol-lowering agent.

Simvastatin Antimicrobial Activity Against Parasites:

Coppens, I., et al. Activity, pharmacological inhibition and biological regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase in Trypanosoma brucei. Mol Biochem Parasitol. 1995a Jan; 69(1): 29-40.

In this study, the authors measured, *in vitro*, the activity of HMG-CoA reductase in the bloodstream form and the culture-adapted procyclic form (insect form) of *Trypanosoma brucei*, the causative agent of sleeping sickness in humans. Synvinolin (simvastatin) was used as a tool to study the regulation of the activity of both HMG-CoA reductase and the abundance of low density lipoprotein (LDL) receptors exposed on the parasite cell surface. In the process, the effect of simvastatin on parasite growth and survival was determined. Simvastatin inhibited the growth of both procyclic and bloodstream forms. In lipoprotein free medium the exponential growth of the procyclics was reduced 2-fold and the sensitivity to synvinolin was enhanced approximately 20-50%. The effect was dose-dependent and increased with time of exposure to the inhibitor (Table 1).

Table 1
IC_{50} (uM) of synvinolin on the growth of *Trypanosoma brucei* and rat foetal fibroblasts in culture

	Bloodstream forms	Procyclic forms		Rat foetal fibroblasts
Lipoproteins in the medium	+	+	-	+
Exponential doubling time(h)	8-9	14	28	22
Incubation time(h)				
24	NT	55±7	39±9	160±25
48	26±4	50±6	27±8	75±9
69	NT	25±8	18±6	51±7

Trypanosomes were grown as described in Materials and Methods in medium containing 10% complete serum (+) or lipoprotein-free serum (-), in the presence of increasing concentrations of synvinolin. At the indicated times, the number of typanosomes was estimated in a haemocytometer, while protein content of adherent fibroblasts was measured by the Lowry assay. Values are means ± SD of IC_{50}, calculated from three separate experiments (NT, not tested).

In addition, growth of procyclics in complete serum showed similar IC_{50} values for 4 other inhibitors tested (compactin, mevinolin, fluvastatin and RG 12561; 53±10 at 40 h, 22±3 uM at 69 h of culture; combined means ±SD). However, growth inhibition due to simvastatin was reversible by products of the mevalonate pathway or by low-density lipoprotein as shown in Table 2 below.

Table 2
Reversal of procyclic growth inhibition due to synvinolin by products of the mevalonate pathway or by low-density lipoprotein

Medium	Growth (% of control)	
	Procyclics	Bloodstream forms
Control	100%	100%
Synvinolin	51±6%	46±3%
Synvinolin + mevalonate (20 mM)	99±7%	88±7%
Synvinolin + squalene (100 uM)	81±5%	41±13%
Synvinolin + cholesterol (100 um)	89±9%	45±11%
Synvinolin + LDL (300 nM)	97±6%	95±7%

Procyclics were first incubated at 28° C in 10% of lipoprotein-free serum, while bloodstream forms were incubated at 37° C in 10% of complete serum, both with or without 25 uM synvinolin, for 40 h. After synvinolin priming, the indicated products of the mevalonate pathway or LDL were added in the medium, and cells were further incubated for 48 h. Finally, the number of trypanosomes was estimated in a haemocytometer. Results are means ± SD of three experiments and expressed in % of control growth, where 100% corresponds to 5.5 10^{-6} ml^{-1} procyclics and 2.5 10^{-6} ml^{-1} bloodstream forms.

Interpretation of these data suggest that synvinolin inhibition of growth is reversed in procyclic forms by mevalonate, squalene, cholesterol and LDL whereas in bloodstream forms growth inhibition is reversed only by mevalonate and LDL.

Coppens, I., et al. Exogenous and endogenous sources of sterols in the culture-adapted procyclic trypomastigotes of Trypanosoma brucei. Mol Biochem Parasitol. 1995b Jul; 73(102): 179-88.

In this paper, the authors extend their work reported in their previous publication. They have demonstrated that procyclics can synthesize their sterols as well as use imported exogenous cholesterol by LDL endocytosis through specific receptors and incorporate this lipid into their membranes. Major changes in the culture medium, such as supplementation with excess LDL, total removal of lipoproteins, or exposure to simvastatin have the capacity to induce modifications in the rate of sterol biosynthesis and in the composition of membranes, as well as modify procyclics' growth rate. These data suggest that procyclics can adapt to extremely different media, so as to maintain a regulated supply of sterols.

Miscellaneous Lovastatin Antimicrobial Activity Studies.

Numerous additional publications with limited information concerning lovastatin antimicrobial activity were identified in the published literature and are cited collectively immediately below this paragraph. The majority of these publications employed lovastatin as a molecular tool in molecular biology studies relative to the elucidation of isoprenoid and steroid biosynthesis mechanisms. Both individually and collectively, these data were not considered as relevant for the purpose of determining reclassification of lovastatin as an antibiotic drug. However, to complete the literature record, they are cited in this report in the event that subsequent discussion, relevant to the consideration of lovastatin's reclassification as an antibiotic, would benefit by their inclusion.

Bard, M., et al. Isolation and characterization of mevinolin resistant mutants of Saccharomyces cerevisiae. J Gen Microbiol. 1988 Apr; 134(Pt4): 1071-8.

Koning, AJ., et al. Different subcellular localization of Saccharomyces cerevisiae HMG-CoA reductase isozymes at elevated levels corresponds to distinct endoplasmic reticulum membrane proliferations. Mol Biol Cell. 1996 May; 7(5): 769-89

Lum, PY., et al. Molecular, functional and evolutionary characterization of the gene encoding HMG-CoA reductase in the fission yeast, Schizosaccharomyces pombe. Yeast. 1996 Sep 15; 12(11): 1107-24.

Ng, WL., et al. Minimal replication origin of the 200-kilobase Halobacterium plasmid pNRC100. J Bacteriol 1993 Aug; 175(15): 4584-96.

Rostand, KS., et al. Cholesterol and cholesterol esters: host receptors for Pseudomonas aeruginosa adherence. J Biol Chem. 1993 Nov 15; 268(32): 24053-9.

Smith, SJ., et al. Transcriptional regulation by ergosterol in the yeast Saccharomyces cerevisiae. Mol Cell Biol. 1996 Oct; 16(10): 5427-32.

Taraboulos, A., et al. Cholesterol depletion and modification of COOH-terminal targeting sequence of the prion protein inhibit formation of the scrapie isoform [published erratum appears in J Cell Biol 1995 Jul: 130(2): 501]. J Cell Biol. 1995 Apr; 129(1): 121-32.

Vanderplasschen, A., et al. The replication in vitro of the gamma herpesvirus bovine herpesvirus 4 is restricted by its DNA synthesis dependence on the S phase of the cell cycle. Virology. 1995 Nov 10; 213(2): 328-40.

DISCUSSION

Data useful for the analysis of whether a drug possesses antimicrobial activity sufficient to warrant its classification as an antibiotic drug product may be obtained from a variety of studies. These studies may include data generated from human clinical trials, animal models and/or from *in vitro* cell cultures. Obviously, data from adequate and well controlled human clinical trials, wherein the antibiotic properties of a drug product have been well characterized, would be the best source of information upon which to base a decision. In the absence of human clinical data, one has two choices with respect to drug classification decision making: 1) determine that the drug is a non-antibiotic drug because relevant human data are unavailable, or 2) utilize preclinical antimicrobial activity data extrapolated to relevant human use circumstances, where possible, in place of human data. Antimicrobial activity associated with lovastatin or related "statin" class of drugs from human clinical studies has not been reported in the literature. Therefore, option 2 has been addressed in this report, recognizing that management may determine a decision based upon option 1.

Ideally, one should have standardized and validated preclinical models for the determination of antimicrobial activity. The term, validation, refers to the circumstances where activity data developed from preclinical models are reproducible and have been shown to be predictive and to correlate with activity subsequently determined in human clinical trials. Unfortunately, the preclinical assays used for generation of antimicrobial data for HMG-CoA reductase inhibitors have been neither standardized nor validated. Consequently, considerable care should be taken when making attempts to determine relevance of preclinical activity data for human drug use parameters.

As a first step in the decision making process for classification of a drug as an antibiotic drug, a clear target definition of antibiotic drug should be determined. As discussed in the background section of this report, the legal definition of an antibiotic drug leaves some room for interpretation from at least two perspectives. First, the species of microorganisms that must be inhibited by a drug product have not been specified. Second, the term "... inhibits in dilute solution ..." does not include an interpretation as to the meaning of "dilute solution." It is recognized that there may be several alternative interpretations applied to this meaning. However, for the purpose of this data analysis and report, the term "... inhibits in dilute solution ..." is interpreted as the drug concentration in preclinical studies that elicits inhibitory activity against microorganisms that correlates with clinically relevant human tissue drug concentrations. Human tissue drug concentrations considered relevant are those that are achieved from doses administered to the human target populations for the indicated use of the drug. The data provided in the Pharmacology section of this report suggest that the target tissue drug concentration of relevance for lovastatin and simvastatin antimicrobial activity should be ~0.1 uM.

Lovastatin and simvastatin *in vitro* antimicrobial activity was evaluated against a variety of bacteria, viruses, yeasts, fungi, and parasites as summarized in Tables 1-3. None of the microorganisms evaluated in these studies was inhibited by concentrations of lovastatin *in vitro* that were $\leq$ 0.1 uM, the target concentration as specified in the definition of dilute solution. However, several different species of microorganisms, including *H. holobium*, *H. volcanii*, HIV, *R. glutinis*, *S. salmonicolor*, *P. blakesleeanus*, *T. cruzi* amastigotes, and *S. mansoni*, were inhibited at 3- to 25-fold greater concentrations than the 0.1 uM target. The remaining microorganisms were inhibited only by lovastatin *in vitro* at concentrations more than 50-fold (range 50- to 1,900-fold) greater than that identified in the target definition of dilute solution. Simvastatin antimicrobial activity was evaluated only in parasites (Table 3), the growth inhibition of which required concentrations at least 125-fold (range 125- to 1,250-fold) greater than the target definition stated above. Reports of pravastatin antimicrobial activity were not found in the published literature.

The majority of the *in vitro* studies described in this report employed an experimental design that utilized minimal media, supplemented with either low concentrations of serum or lipoprotein depleted serum, for microorganism growth. This fact imposes a serious limitation upon interpretation of these data with respect to potential *in vivo* lovastatin antimicrobial activity. For example, *in vitro* growth inhibitory effects of the HMG-CoA reductase inhibitor, compactin, on Chinese hamster ovary cells (CHO) were shown to be dependent upon the amount of low density lipoprotein (LDL) and mevalonate present in the growth medium (Goldstein, et al., 1979; cited in a review by Brown and Goldstein, 1980). They reported that in the presence of either 2 uM or 40 uM compactin and in the absence of both LDL and mevalonate, CHO cells failed to grow. On the other hand, growth inhibition of cells treated with 2 uM compactin was reversed by the addition of 25 ug/ml LDL, but not by the addition of 0.5 mM mevalonate, to the culture medium. When cells were treated with 40 uM compactin, neither 25 ug/ml LDL alone nor 0.5 mM mevalonate supported growth. However, the combination of 25 ug/ml LDL and 0.5 mM mevalonate restored full growth of CHO cells even in the presence of 40 uM compactin. Interpretation of these data shows that the MIC of compactin can be increased by a minimum of 20-fold (i.e., 2 uM to 40 uM), and perhaps more, depending upon the composition of the growth medium with respect to LDL and mevalonate content. The concentrations of LDL and mevalonate necessary to reverse HMG-CoA reductase inhibition of cell growth are variable. In the absence of cholesterol, cell growth requires large amounts of mevalonate, most of which is channeled into cholesterol biosynthesis. When cholesterol is present in saturating amounts, only a small amount of mevalonate, required for isoprenoid biosynthesis, is necessary to support cell growth. In many of the publications reviewed for this report, the antimicrobial activity of lovastatin was shown to be reversed by the addition of varying amounts of mevalonate or other products of the steroid or isoprenoid biosynthetic pathways, such as LDL, cholesterol, farnesol, and squalene. Moreover, reversal of growth inhibition required less mevalonate in studies that employed higher level of serum in their growth medium. Interpretations from these data suggest that antimicrobial activity of HMG-CoA reductase inhibitors determined from these *in vitro* study results would be substantially diminished if the assay media employed in these studies were not limited in serum and contained concentrations of mevalonate and LDL normally present *in vivo*. These observations would suggest that HMG-CoA reductase inhibitors should exhibit significantly less antimicrobial activity *in vivo* than that observed in these *in vitro* studies.

Only four murine animal model *in vivo* studies containing lovastatin antimicrobial activity data were identified in the literature; two studies on *S. mansoni* and two on *T. cruzi*. In *S. mansoni*, adult schistosome survival was reported to be reduced 96-100% in mice fed 0.2% lovastatin (640 mg/kg) in their diet for 14 days (Chen, et al., 1990). At 250 mg/kg, adult schistosome survival was reported to be unaffected although egg production was inhibited 45.4% (Vandewaa, et al., 1989). In mice treated with 100 mg/kg lovastatin, egg production was unaffected while at 50 mg/kg egg production was enhanced. Thus, it appears that considerably high levels of lovastatin are required to inhibit *S. mansoni* adult schistosomes and egg production *in vivo*, in spite of the *in vitro* sensitivity of *S. mansoni* also reported in these studies.

Conflicting data were reported concerning lovastatin's antimicrobial activity against *T. cruzi* infection in mice. Urbina, et al., 1993, reported that lovastatin, as a single drug, was incapable of inhibiting parasitemia in infected mice, although a dose of 20 mg/kg/day administered for 7 days increased survival 50% over untreated, infected, control mice. In contrast, Brener, et al., 1993, reported that lovastatin at 100 mg/kg/day exacerbated parasitemia approximately 3-fold

over untreated controls and failed to provide a survival benefit. These data are in contrast to results obtained from *in vitro* studies where, at least for *T. cruzi* amastigotes, growth inhibitory concentrations were only 10-fold greater than the target definition of "... inhibits in dilute solution . .." Lovastatin, at 1 uM, was reported to eliminate *T. cruzi* amastigotes from *in vitro* cell cultures within 192 h; whereas, epimastigotes required concentrations of 25 to 125 uM (Urbina, et al., 1993; Florin-Christensen, et al., 1990). These results are consistent with the prediction that *in vivo* antimicrobial activity of HMG-CoA reductase inhibitors would be substantially less than that reported from the *in vitro* studies.

Several publications also contained data on the antimicrobial effect of lovastatin in combination with azoles. Azoles are known to inhibit ergosterol biosynthesis in yeasts and fungi. The rationale for the study design was two-fold. First, lovastatin had been reported to inhibit sterol biosynthesis in the yeast, *S. cerevistae*, and in the process increase cell membrane permeability to exogenous sterols. It was anticipated that this increase in cell permeability would extend to azoles present in the cultures, thus, potentiating antimicrobial activity of the azole. Secondly, lovastatin and azoles inhibit two separate enzymes involved in the biosynthetic pathway of ergosterol, a sterol required for growth of fungi, yeasts and some parasites. Exposure of a microorganism to drugs capable of inhibiting two separate targets in ergosterol biosynthesis was anticipated to be able to maintain antimicrobial activity of the azole while permitting lower, perhaps non-toxic, doses of azoles to be used in the treatment of infections.

In general, results from *in vitro* studies showed that lovastatin in combination with azole drugs resulted in a synergistic antimicrobial interaction against several microorganisms. However, the same cautions pertaining to the *in vitro* lovastatin antimicrobial data referred to above should be applied to these combination studies. In addition, combination drug activity observed in a murine model of parasite infection was less impressive with respect to antiparasitic effects. One report suggested a slight reduction in ketoconazole required to eliminate *T. cruzi* parasitemia in mice when used in combination with lovastatin (Urbina, et al., 1993). However, a separate report suggested an antagonistic interaction for lovastatin and ketoconazole when used in combination against the same species of microorganism (Brener, et al., 1993).

Unfortunately, the *in vivo* results reported in the above studies are further complicated by the fact that the authors failed to take into consideration a very major concern. Ketoconazole and itraconazole are known to inhibit the cytochrome P450 3A enzyme family responsible for the metabolism of lovastatin (Wang, et al., 1991; Back, et al., 1992; Rotstein, et al., 1992). Inhibition of this enzyme by itraconazole has been shown to increase the concentration of lovastatin by 20- to 30-fold in normal human subjects administered 200 mg itraconazole daily for 4 days followed by a single 40 mg dose of lovastatin on day four (Neuvonen, et al., 1996). In one of the 12 subjects in the study, creatine phosphokinase increased 10-fold within 24 hours following administration of the lovastatin dose, indicating skeletal muscle toxicity. This increase did not occur when the subject was given the same lovastatin dose four weeks later without itraconazole. Moreover, in transplant patients taking lovastatin and cyclosporine, a drug that inhibits cytochrome P450 enzyme CYP 3A4, serious myopathies (attributed to increased plasma lovastatin concentrations) have been reported that can be controlled by lovastatin dose reduction and careful monitoring of lovastatin plasma levels (Arnadottir, et al., 1993).

While the authors of the studies for the evaluation of antimicrobial activity associated with lovastatin in combination with ketoconazole focused on the potential to reduce interference with hepatic function and testosterone production associated with high doses of ketoconazole, they failed to consider the effects of ketoconazole on increasing tissue lovastatin concentrations and the potential for lovastatin induced toxicity exacerbation. It is not clear from the data available if lovastatin concentrations, when reduced sufficiently to avoid potential toxicity reactions, would elicit a synergistic response with respect to ketoconazole's antiparasitic activity to be meaningful Moreover, the concept of lovastatin's ability to potentiate the activity of another drug that is not an antibiotic may be irrelevant to the discussion.

Table 1. Summary of Lovastatin *in vitro* Activity Against Bacteria and Viruses.

Microorganism Inhibited	Inhibitory Concn. (uM)	Reference	Comments
Escherichia coli	>68.3	Zhou, et al., 1991	Inhibition of growth was not achieved. Eubacteria do not utilize acetyl CoA and mevalonate in biosynthesis of isoprenoids.
Halobacterium holobium	1-2	Cabrera, et al., 1986	Cells of the genus, *Halobacterium*, require >15% NaCl for growth. Inhibition reversed by 4 mM mevalonate.
Halobacterium volcanii	1-2 20-40	Lam, et., 1989	In minimal medium, MIC is 1-2 uM. In enriched medium, MIC is 20-40 uM.
Murine Leukemia Virus (MuLV)	ND*	Overmeyer, 1992	2.5 uM lovastatin prevented maturation of MuLV's glycoprotein precursor, $gPr90^{env}$, to the mature envelope glycoprotein, $gp70^{env}$. Inhibition of virus infectivity was not reported.
Human Immunodeficiency Virus (HIV)	0.3	Maziere, et al., 1994	H9 cells were adapted to grow in medium supplemented with 1% serum to limit exogenous cholesterol. Virus inhibition was determined by a reverse transcriptase assay. Reverse transcriptase was reduced ~10-fold after lovastatin treatment compared to untreated, infected controls.
Measles Virus	ND	Malvoisin, et al., 1990	Measles virus induced syncytia in Vero cells was inhibited at ~15 uM lovastatin. Inhibition of measles virus infectivity was not reported.

*Not determined.

Table 2. Summary of Lovastatin *in vitro* Activity Against Yeasts and Fungi

Microorganism Inhibited	Inhibitory Concn. (uM)	Reference	Comments
Rhodotorula glutinis	~0 25	Ikeura, et al., 1988	Cells were grown in 0.67% yeast nitrogen base with 0 5% glucose. Cell inhibition with compactin was shown to be reversed, except for *C. matritensis*, by addition of 10 mM mevalonate to the culture medium.
Sporobolomyces salmonicolor	~2.5		
Aessosporon salmonicolor	~5.0[a]		
Citeromyces matritensis	~5.0[a]		
Saccharomyces cerevisiae	≥190	Lorenz, et al., 1990	Lovastatin at ~ 25 uM, in combination with ketoconazole, clotrimazole or miconazole, decreased the MICs of these azoles 6-, 10- and 32-fold, respectively, suggesting a synergistic antimicrobial activity between lovastatin and azoles against *S. cerevisiae*.
Candida albicans VA	~125	Sud, et al, 1985	Lovastatin, at concentrations between ~2 and 62.5 uM, gave a fourfold or greater reduction in ketoconazole MICs when used in combination studies. However, a fourfold reduction for *C. tropicalis* and *T. glabrata* was not obtained. These data were generated utilizing completely synthetic media.
Candida albicans 7.22	~250		
Candida tropicalis	>250		
Torulopsis glabrata	>250		
Aspergillus fumigatus 173	~15		
Aspergillus fumigatus	~15		
Aspergillus niger	~30		
Rhizopus rhizopodiformis	~125		
Phycomyces blakesleeanus	1	Bejarano, et al., 1992	Fungus was grown on minimal agar medium. The observed inhibition by lovastatin was reversed by the presence of 10 mM but not 1 mM mevalonate.
Physarum polycephalum	≥25	Engstrom, et al., 1989	Inhibition of protein synthesis, DNA synthesis, nuclear division and plasmodia growth could be partially reversed by the addition of mevalonate at concentrations ≥ 0.4 mM.

[a]Inhibition determined with compactin only (lovastatin is an analogue of compactin).

Table 3. Summary of Lovastatin and Simvastatin *in vitro* Activity Against Parasites

Microorganism Inhibited	Inhibitory Concn. (uM)	Reference	Comments
Trypanosoma cruzi	25 to 125	Florin-Christensen, et al., 1990	Lovastatin inhibited growth in a dose dependent fashion; at 25 and 75 uM, growth was progressively inhibited. However, 125 uM was required to kill most of the trypanosomes. Squalene (100 uM), but not cholesterol, was able to reverse growth inhibition produced by 25 to 75 uM lovastatin.
Trypanosoma cruzi			
epimastigotes	50 to 75	Urbina, et al., 1993	Growth was reduced 20-30% at lovastatin concentrations of 7.5 uM. Complete inhibition was observed at 50 and 75 uM after 144 h and 96 h of culture incubation, respectively. Trypanocidal concentration of lovastatin was reduced by a factor of 10 when incubated in combination with 0.1 uM ketoconazole.
amastigotes	>1		Concentrations of lovastatin greater than 1 uM were cidal for the Vero cells used to maintain the amastigotes in culture. Only 30% growth inhibition was observed for the amastigote at 1 uM lovastatin concentration. Lovastatin 0.75 uM in combination with 1 nM ketoconazole eliminated amastigotes from cell cultures after 192 h of incubation. Terbinifine 1 uM, required 25 uM lovastatin for complete growth inhibition.
Leishmania donovani			
promastigotes	~200	Haughan, et al., 1992	*L. amazonensis* was the strain most sensitive to lovastatin. In combination with miconazole, the IC_{50} values of each drug could be reduced 2- to 10-fold compared to when used as a single agents. At 25 uM, lovastatin had little effect on amastigotes in macrophage culture. Due to drug solubility problems, higher concentrations could not be tested.
Leishmania amazonensis			
promastigotes	~50		
amastigotes	>25		
Schistosoma mansoni	>100	Morrison, et al., 1986	Egg production in *S. mansoni* grown *in vitro* with 50% horse serum could be depressed ~50% at 1 uM lovastatin. However, at 100 uM complete inhibition of egg production was not obtained and growth inhibition of adult mating pairs was minimal. Egg production inhibition was not reversed by coincubation with 100 uM cholesterol. Reversal by mevalonate was not evaluated.
Schistosoma mansoni	10	Vandewaa, et al., 1989	Egg production in *S. mansoni* grown *in vitro* with 50% horse serum was inhibited ~5-fold at 10 uM lovastatin. Inhibition by lovastatin could be reversed by the addition of either farnesol or mevalonate at a concentration of 80 uM.
Schistosoma mansoni	1 to 10	Chen, et al., 1990	Lovastatin inhibition of adult schistosome motility and lactate production was time and dose dependent. At 3 days incubation, 10 uM lovastatin reduced motility and lactate production >50%; at 11 days of culture, doses of 1-10 uM inhibited activity nearly 90%. Inhibition was reversed in the presence of 50 uM mevalonate.
Giardia lamblia	≥200	Lujan, et al., 1995	Protein isoprenylation and cell growth were inhibited in a dose dependent manner with complete inhibition obtained by concentrations of compactin and mevinolin ≥200 uM. Data for mevinolin were not shown. The inhibition could be reversed by the addition of 2 mM mevalonate to the culture medium.
Plasmodium falciparum			
F32/Tanzania	>125[b,c]	Grellier, et al., 1994	IC_{50} values for *P. falciparum* were in the range of 25 to 50 uM. However, complete inhibition of growth was observed only with concentrations >125 uM. Simvastatin was tested against these organisms with similar results obtained.
FcB/Columbia	>125[b,c]		
Babesia divergens			
Rouen 1987	~12.5 to 25[c]	Grellier, et al., 1994	Activity of lovastatin was not determined against the Weybridge strain.
Weybridge 8843	~15[b]		
Trypanosoma brucei brucei	>20	Andersson, et al, 1996	Lovastatin, at a concentration of 0.1 uM, blocked interferon gamma induced proliferative responses of *T. brucei brucei*. However, growth of non-stimulated parasites was not affected at concentrations as high as 20 uM.
Trypanosoma brucei			
bloodstream forms	25[b]	Coppens, et al., 1995a	Simvastatin inhibited the growth of both bloodstream and procyclic forms of *T. brucei* with IC_{50} values in the range of 25 uM and 18 to 55 uM, respectively. In lipoprotein free medium the exponential growth of the procyclics was reduced 2-fold and the sensitivity to simvastatin was enhanced approximately 20-50%.
procyclic forms	18 to 55[b] 22 to 53[c]		

[a]Not determined.
[b]Reported for simvastatin.
[c]Reported for lovastatin

Table 4. Summary of Lovastatin *in vivo* Activity Against Parasites

Microorganism Inhibited	Inhibitory Concn. (mg/kg)	Reference	Comments
Schistosoma mansoni	>250	Vandewaa, et al., 1989	Adult schistosomes were unaffected in mice treated with 250 mg/kg lovastatin orally for 10 days. Egg production was inhibited 45.4% in these mice. However, in mice treated with 50 mg/kg, egg production was enhanced (degree of enhancement not reported) over that observed in control mice.
Schistosoma mansoni	640	Chen, et al., 1990	Adult schistosome survival was reduced 96-100% in mice fed a diet consisting of 0.2% lovastatin (640 mg/kg/day) for 14 days.
Trypanosoma cruzi	ND*	Urbina, et al., 1993	Lovastatin as a single drug was incapable of inhibiting parasitemia in infected mice. However, at a dose of 20 mg/kg/day administered for 7 days, mouse survival was promoted 50% over untreated controls. When a low dose of lovastatin and ketoconazole were combined, 100% survival and almost complete suppression of parasitemia were reported.
Trypanosoma cruzi	ND	Brener, et al , 1993	At a dose of 100 mg/kg/day administered for 19 days post-infection, lovastatin exacerbated parasitemia approximately 3-fold over untreated controls and failed to provide a survival benefit associated with treatment. In combination studies with ketoconazole, lovastatin appeared to elicit an antagonistic response with respect to ketoconazole's antiparasitic activity.

*Not determined.

CONCLUSIONS

Lovastatin, simvastatin, and pravastatin are the only anti-hypercholesteolemia drug products in CDER that meet the part of the antibiotic drug definition "... produced by microorganisms or any chemically synthesized equivalent ...".

Antimicrobial activity associated with lovastatin, simvastatin, and pravastatin in humans studies was not found in the published literature.

Antimicrobial activity associated with pravastatin was not found in the published literature.

Antimicrobial activity associated with lovastatin and simvastatin from *in vitro* and *in vivo* studies was reported.

The concentration of lovastatin and simvastatin in plasma obtained from human subjects administered the maximum approved dose daily for 17 days, the target parameters relevant for the antibiotic drug definition "... inhibits in dilute solution ...", was estimated to be ~0.1 uM.

None of the bacteria, viruses, yeasts, fungi, or parasites evaluated in the *in vitro* studies conducted for the assessment of antimicrobial activity was inhibited by lovastatin or simvastatin concentrations of 0.1uM.

Several species of microorganisms were inhibited at concentrations of lovastatin 3- to 25-fold greater than the target lovastatin tissue concentration of 0.1 uM. The remainder were reported to be inhibited at concentrations of 50- to 1,900-fold greater than 0.1uM.

The majority of the *in vitro* studies utilized assays that severely restricted serum and lipoprotein. Growth inhibition by HMG-CoA reductase inhibitors is known to be significantly enhanced when assayed in limited serum or lipoprotein conditions.

Growth inhibition can be reversed by the addition of LDL and mevalonate to cultures.

These facts suggest that the *in vitro* assays used in these studies are artificial systems and that the antimicrobial activity observed for lovastatin and simvastatin in these assays would be substantially diminished in an *in vivo* environment.

As predicted, lovastatin antimicrobial activity in a murine model of *Schistosoma mansoni* and *Trypanosoma cruzi* infections was reported to be minimal

If the target human tissue lovastatin and simvastatin concentration of 0.1 uM is used as a basis for the definition of "... inhibits in dilute solution ...", the available data are insufficient to support the conclusion that lovastatin, simvastatin, and pravastatin have sufficient antimicrobial activity to warrant their reclassification as antibiotic drugs.

LITERATURE CITED

Alberts, AW. Lovastatin and simvastatin--inhibitors HMG CoA reductase and cholesterol biosynthesis. Cardiology. 1990; 77 Suppl 4: 14-21.

Alberts, AW. Discovery, biochemistry and biology of lovastatin. Am J Cardiol. 1988 Nov 11; 62(15): 10J-15J.

Alberts, AW., et al. Mevinolin: a highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol-lowering agent. Proc Natl Acad Sci. 1980; 77: 3957-61.

Andersson, M., et al. Lovastatin inhibits interferon-gamma-induced Trypanosoma brucei proliferation: evidence for mevalonate pathway involvement. J Interferon Cytokine Res. 1996 Jun; 16(6): 435-9.

Arnadottir, M., et al. Plasma concentration profiles of simvastatin 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitory activity in kidney transplant recipients with and without ciclosporin. Nephron. 1993; 65(3): 410-3.

Back, et al. Azoles, allylamines and drug metabolism. Br J Dermatol. 1992 Feb; 126 suppl 39:14-18.

Bard, M., et al. Isolation and characterization of mevinolin resistant mutants of Saccharomyces cerevisiae. J Gen Microbiol. 1988 Apr; 134(Pt4): 1071-8.

Bejarano, ER., et al. Independence of the carotene and sterol pathways of Phycomyces. FEBS Lett. 1992 Jul 20; 306(2-3): 209-12.

Brener, Z., et al. An experimental and clinical assay with ketoconazole in the treatment of Chagas disease. Mem Inst Oswaldo Cruz. 1993 Jan-Mar; 88(1): 149-53.

Brown, MS., et al. Multivalent feedback regulation of HMG CoA reductase, a control mechanism coordinating isoprenoid synthesis and cell growth. J Lipid Res. 1980; 21: 505-517.

Brown, AG., et al. Crystal and molecular structure of compactin, a new antifungal metabolite from Penicillium brevicompactum. J Chem Soc Perkin I. 1976; 11:1165-1170.

Cabrera, JA., et al. Isoprenoid synthesis in Halobacterium holobium. Modulation of 3-hydroxy-3-methylglutaryl coenzyme A concentration in response to mevalonate availability. J Biol Chem. 1986 Mar 15; 261(8): 3578-83.

Chen, GZ., et al. Antischistosomal action of mevinolin: evidence that 3-hydroxy-methylglutaryl-coenzyme A reductase activity in Schistosoma mansoni is vital for parasite survival. Naunyn Schmiedebergs Arch Pharmacol. 1990 Oct; 342(4): 477-82.

Coppens, I., et al. Activity, pharmacological inhibition and biological regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase in Trypanosoma brucei. Mol Biochem Parasitol. 1995a Jan; 69(1): 29-40.

Coppens, I., et al. Exogenous and endogenous sources of sterols in the culture-adapted procyclic trypomastigotes of Trypanosoma brucei. Mol Biochem Parasitol. 1995b Jul; 73(102): 179-88.

Desager, JP., et al. Clinical pharmacokinetics of 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors. Clin Pharmacokinet. 1996 Nov; 31(5): 348-71.

Duggan, DE., et al. The physiological disposition of lovastatin. Drug Metab Dispos. 1989 Mar-Apr; 17(2): 166-73.

Endo, AM., et al. ML-236A, ML-236B, and ML-236C, new inhibitors of cholesterogenesis produced by Penicillium citrinum. J Antibiot (Japan). 1976a; 29: 1346-1348.

Endo, A., et al. Competitive inhibition of 3-hydroxy-3-methyl-glutaryl coenzyme A reductase by ML-236A and ML-236B, fungal metabolites having hypocholesterolemic activity. FEBS Lett. 1976b; 72: 323-326.

Endo, A., et al. Chemistry, biochemistry, and pharmacology of HMG-CoA reductase inhibitors. Klin Wochenschr. 1988 May 16; 66(10): 421-7.

Endo, A. Drugs inhibiting HMG-CoA reductase. Pharmac Ther. 1985a; 31(3): 257-67.

Endo, A., et al. Microbial phosphorylation of compactin (ML-236B) and related compounds. J Antibiot Tokyo. 1985b Mar; 38(3): 328-32.

Endo, A. The discovery and development of HMG-CoA reductase inhibitors. J Lipid Res. 1992 Nov; 33(11): 1569-82.

Engstrom, W., et al. The effects of tunicamycin, mevinolin and mevalonic acid on HMG-CoA reductase activity and nuclear division in the myxomycete Physarum polycephalum. J Cell Sci. 1989 Mar; 92(Pt 3): 341-4.

Florin-Christensen, M., et al. Inhibition of Trypanosoma cruzi growth and sterol biosynthesis by lovastatin. Biochem Biophys Res Commun. 1990 Feb 14; 166(3): 1441-5.

Germershausen, JI., et al. Tissue selectivity of the cholesterol-lowering agents lovastatin, simvastatin and pravastatin in rats in vivo [published erratum appears in Biochem Biophys Res Commun 1991 Jan 31: 174(2):1053]. Biochem Biophys Res Commun. 1989 Feb 15; 158(3): 667-75.

Grellier, P., et al. 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors lovastatin and simvastatin inhibit in vitro development of Plasmodium falciparum and Babesia divergens in human erythrocytes. Antimicrob Agents Chemother. 1994 May; 38(5): 1144-8.

Gunde-Cimerman, N., et al. Pleurotus fungi produce mevinolin, and inhibitor of 3-hydroxy-3-methylglutaryl Coenzyme A reductase-lovastatin. FEMS Microbiol Lett. 1993 Nov 1; 113(3): 333-7.

Halpin, RA., et al. Biotransformation of lovastatin. V. Species differences in in vivo metabolite profiles of mouse, rat, dog, and human. Drug Metab Dispos. 1993 Nov-Dec; 21(6): 1003-11.

Haughan, PA., et al. Synergism in vitro of lovastatin and miconazole as anti-leishmanial agents. Biochem Pharmacol. 1992 Dec 1; 44(11): 2199-206.

Ikeura, R., et al. Growth inhibition of yeast by compactin (ML-236B) analogues. J Antibiot Tokyo. 1988 Aug; 41(8): 1148-50.

Jungnickel, PW., et al. Pravastatin: a new drug for the treatment of hypercholesterolemia [see comments]. Clin Pharm. 1992 Aug; 11(8): 677-89.

Kaneko, I., et al. Inhibitory effects of lipid metabolism in cultured cells of ML-236B, a potent inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase. Eur J Biochem. 1978, 87: 313-321.

Koning, AJ., et al. Different subcellular localization of Saccharomyces ceresvisiae HMG-CoA reductase isozymes at elevated levels corresponds to distinct endoplasmic reticulum membrane proliferations. Mol Biol Cell. 1996 May; 7(5): 769-89.

Lam, WL., et al Shuttle vectors for the archaebacterium Halobacterium volcanii. Proc Natl Acad Sci USA. 1989 Jul; 86(14): 5478-82.

Lorenz, RT.,et al. Effects of lovastatin (mevinolin) on sterol levels and on activity of azoles in Saccharomyces cerevisiae. Antimicrob Agents Chemother. 1990 Sep; 34(9): 1660-5.

Lujan, HD., et al. Isoprenylation of proteins in the protozoan Giardia lamblia. Mol Biochem Parasitol. 1995 Jun; 72(1-2): 121-7.

Lum, PY., et al. Molecular, functional and evolutionary characterization of the gene encoding HMG-CoA reductase in the fission yeast, Schizosaccharomyces pombe. Yeast. 1996 Sep 15; 12(11): 1107-24.

Malvoisin, E., et al. Effect of drugs which inhibit cholesterol synthesis on syncytia formation in vero cells infected with measles virus. Biochim Biophys Acta. 1990 Feb 23; 1042(3): 359-64.

Maziere, JC., et al. Lovastatin inhibits HIV-1 expression in H9 human T lymphocytes cultured in cholesterol-poor medium. Biomed Pharmacother. 1994; 48(2): 63-7.

Moore, RN., et al. Biosynthesis of the hypocholesterolemic agent mevinolin by Aspergillus terreus. Determination of the origin of carbon, hydrogen, and oxygen atoms by ^{13}C NMR and mass spectrometry. J Am Chem Soc. 1985; 107: 3694-3701.

Morrison, DD., et al. Effects of steroids and steroid synthesis inhibitors on fecundity of S. mansoni in vitro. J Chem Ecol. 1986; 12: 1901-08.

Neuvonen, PJ., et al. Itraconazole drastically increases plasma concentrations of lovastatin and lovastatin acid. Clin Pharmacol Ther. 1996 Jul; 60(1): 54-61.

Overmeyer, JH. Isoprenoid requirement for intracellular transport and processing of murine leukemia virus envelope protein. J Biol Chem. 1992 Nov 5; 267(31): 22686-92.

Rostand, KS., et al. Cholesterol and cholesterol esters: host receptors for Pseudomonas aeruginosa adherence. J Biol Chem. 1993 Nov 15; 268(32): 24053-9.

Rotstein, DM., et al. Stereoisomers of ketoconazole: preparation and biological activity. J Med Chem. 1992 Jul 24; 35(15): 2818-25.

Sirtori, CR. Pharmacology and mechanism of action of the new HMG-CoA reductase inhibitors Pharmacol Res. 1990 Sep-Oct; 22(5): 555-63.

Smith, SJ., et al. Transcripional regulation by ergosterol in the yeast Saccharomyces cerevisiae. Mol Cell Biol. 1996 Oct; 16(10): 5427-32.

Sud, IJ., et al. Effect of ketoconazole in combination with other inhibitors of sterol synthesis on fungal growth. Antimicrob Agents Chemother. 1985. 28: 532-534.

Taraboulos, A., et al. Cholesterol depletion and modification of COOH-terminal targeting sequence of the prion protein inhibit formation of the scrapie isoform [published erratum appears in J Cell Biol 1995 Jul: 130(2): 501]. J Cell Biol. 1995 Apr; 129(1): 121-32.

Tobert, JA. New developments in lipid-lowering therapy: the role of inhibitors of hydroxymethylglutaryl-coenzyme A reductase. Circulation 1987 Sep; 76(3): 534-8.

Tsujita, Y., et al. CS-514, a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase: tissue-selective inhibition of sterol synthesis and hypolipidemic effect on various animal species. Biochim Biophys Act. 1986 Jun 11; 877(1): 50-60.

Urbina, JA., et al. Mevinolin (lovastatin) potentiates the antiproliferative effects of ketoconazole and terbinafine against Trypanosoma (Schizotrypanum) cruzi: in vitro and in vivo studies. Antimicrob Agents Chemother. 1993 Mar; 37(3): 580-91.

Vanderplasschen, A., et al. The replication in vitro of the gamma herpesvirus bovine herpesvirus 4 is restricted by its DNA synthesis dependence on the S phase of the cell cycle. Virology. 1995 Nov 10; 213(2): 328-40.

Vandewaa, EA., et al. Physiological role of HMG-CoA reductase in regulating egg production by Schistosoma mansoni. Am J Physiol 1989 Sep; 257(3 Pt 2): R618-25.

Vyas, KP., et al. Biotransformation of lovastatin. I. Structure elucidation of in vitro and in vivo metabolites in the rat and mouse. Drug Metab Dispos. 1990a Mar-Apr; 18(2): 203-11.

Vyas, KP., et al Biotransformation of lovastatin. II. In vitro metabolism by rat and mouse liver microsomes and involvement of cytochrome P-450 in dehydrogenation of lovastatin. Drug Metab Dispos.. 1990b Mar-Apr; 18(2): 218-22.

Wang, RW., et al. Biotransformation of lovastatin. IV. Identification of cytochrome P450 3A proteins as the major enzymes responsible for the oxidative metabolism of lovastatin in rat and human liver microsomes. Arch Biochem Biophys. 1991 Nov 1; 290(2): 355-61.

Zhou, D., et al. Early steps of isoprenoid biosynthesis in Escherichia coli. Biochem J. 1991 Feb 1; 273(Pt 3): 627-34.

Additional Background Reading

Aggarwal, D., et al. A quantitative structure-activity relationship study of 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitors J Pharmacobiodyn 1988 Sep; 11(9): 591-9

Amin, D., et al. RPR 101821, a new potent cholesterol-lowering agent: inhibition of squalene synthase and 7-dehydrocholesterol reductase. Naunyn-Schmiedebergs-Arch-Pharmacol. 1996 Jan; 353(2): 233-40.

Ansari, S., et al. Effect of miconazole on the structure and function of plasma membrane of Candida albicans. FEMS Microbiol Lett. 1993 Nov 15; 114(1): 93-8.

Bard, M., et al. Genetic and biochemical aspects of yeast sterol regulation involving 3-hydroxy-3-methylglutaryl coenzyme A reductase. J Gen Microbiol. 1981; 125: 415-420.

Basson, ME., et al. Identifying mutations in duplicated functions in Saccharomyces cerevisiae: recessive mutations in HMG-CoA reductase genes. Genetics. 1987; 117: 645-55.

Basson, ME., et al. Saccharomyces cerevisiae contains two functional genes encoding 3-hydroxy-3-methylglutaryl coenzyme A reductase. Proc Natl Acad Sci USA. 1986; 83: 5563-5567.

Beach, MJ., et al. Cloning, sequencing, and overexpression of mvaA, which encodes Pseudomonas mevalonii 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase. J Bacteriol. 1989; 171: 2994-3001.

Berens, KL., et al. Influence of lovastatin on the pharmacokinetics, toxicity and immunologic response of cyclosporine in the obese Zucker rat. Biopharm Drug Dispos. 1990 Apr; 11(3): 197-206.

Bischoff, KM., et al. 3-Hydroxy-3-methylglutaryl-coenzyme A reductase from Haloferax volcanii: purification, characterization, and expression in Escherichia coli. J Bacteriol. 1996 Jan; 178(1): 19-23

Blum, CB. Comparison of properties of four inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase [published erratum appears in Am J Cardiol. 1994 Sep 15;74(6):639]. Am J Cardiol. 1994 May 26; 73(14): 3D-11D.

Bocan, TM., et al. Hepatic and nonhepatic sterol synthesis and tissue distribution following administration of a liver selective HGM-CoA reductase inhibitor, CI-981: comparison with selective HMG-CoA reductase inhibitors. Biochim Biophys Acta 1992 Jan 24; 1123(2): 133-44.

Casolari, C., et al. Keratomycosis with an unusual etiology (Rhodotorula glutinis): a case report. Microbiologica 1992 Jan; 15(1): 83-7

Chao, TY., et al. Diagnosis of fungemia in bone marrow transplantation patients by examination of peripheral blood smears. Bone Marrow Transplant. 1994 Oct; 14(4): 647-9.

Chen, GZ., et al. Purification and characterization of 3-hydroxymethylglutaryl-coenzyme A reductase of Schistosoma mansoni: regulation of parasite enzyme activity differs from mammalian host. Exp Parasitol. 1991 Jul; 73(1): 82-92.

Cheung, AK., et al. A prospective study on treatment of hypercholesterolemia with lovastatin in renal transplant patients receiving cyclosporine. J Am Soc Nephrol. 1993 Jun; 3(12): 1884-91.

Cutts, JL., et al. Suppression of lymphoid cell function in vitro by inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase by lovastatin. Int J Immunopharmacol. 1989; 11(8): 863-9.

Cutts, JL., et al. Role of mevalonic acid in the regulation of natural killer cell cytotoxicity. J Cell Physiol. 1989 Jun; 139(3): 550-7.

Dietschy, JM., et al. Regulation of cholesterol metabolism. N Engl J Med. 1970; 282: 1128-1138.

Doi, I., et al. Specific inhibition of desmosterol synthesis by ML-236B in mouse LM cells grown in suspension in a lipid-free medium. J Med Sci Biol (Japan). 1978; 31: 225-233.

Downing, JF., et al. The isolation of two mutants of Saccharomyces cerevisiae which demonstrate increased activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase. Biochem Biophys Res Commun. 1980; 94: 974-979.

Duane, WC. Effects of lovastatin and dietary cholesterol on bile acid kinetics and bile lipid composition in healthy male subjects. J Lipid Res. 1994 Mar; 35(3): 501-9.

Duane, WC. Effects of lovastatin in humans on biliary lipid composition and secretion as a function of dosage and treatment interval. J Pharmacol Exp Ther. 1994 Sep; 270(3): 841-5.

Dyall-Smith, ML., et al. Construction of composite transposons for halophilic Archaea. Can J Microbiol. 1994 Nov; 40(11): 922-9.

Field, H., et al. Characterisation of protein isoprenylation in procyclic form Trypanosoma brucei. Mol Biochem Parasitol. 1996 Nov 12; 82(1): 67-80.

Field, H., et al. Protein isoprenylation in Trypanosoma brucei. Biochem, Soc Trans. 1996 Aug; 24(3): 443S.

Fini, A., et al. Quantitative structure-antimicrobial activity relationship in 5 beta-cholanyl-24-benzylamine derivatives. J Pharm Sci. 1990 Jul; 79(7): 603-5.

Gerson, RJ., et al. Animal safety and toxicology of simvastatin and related hydroxy-methylglutaryl-coenzyme A reductase inhibitors. Am J Med. 1989 Oct 16; 87(4A): 28S-38S.

Giannakouros, T., et al. Protein prenylation in Schizosaccharomyces pombe. FEBS Lett. 1992 Feb 3; 297(1-2: 103-6.

Gill, JF Jr., et al. Transport of mevalonate by Pseudomonas sp. Strain M. J Bacteriol. 1984 Oct; 160(1): 294-8.

Gunde-Cimerman, N., et al. Pleurotus fruiting bodies contain the inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase-lovastatin. Exp Mycol. 1995 Mar; 19(1): 1-6.

Glomset, JA., et al. The prenylation of proteins. Curr Opin Lipidol. 1992, 118-124.

Goldstein, JL., et al. Regulation of the mevalonate pathway. Nature. 1990; 343: 425-430.

Guerra, R., et al. Rhodotorula glutinis karatitis. Int Ophthalmol. 1992 May; 16(3): 187-90.

Gyaurgieva, OH., et al. Meningitis caused by Rhodotorula rubra in an HIV-infected patient. J Med Vet Mycol. 1996 Sep-Oct; 34(5): 357-9.

Hallander, HO., et al. Synergism between aminoglycosides and cephalosporin with antipseudomonal activity: interaction index and killing curve method. Antimicrob Agents Chemother. 1982; 22: 743-752.

Haruyama, H., et al. Structure elucidation of the bioactive metabolities of ML-236B (mevastatin) isolated from dog urine. Chem Pharm Bull Tokyo. 1986 Apr; 34(4): 1459-67.

Hazen, KC. New and emerging yeast pathogens. Clin Microbiol Rev. 1995 Oct; 8(4): 462-78

Henwood, JM , et al. Lovastatin, a preliminary review of its pharmacodynamic properties and therapeutic use in hyperlipidemia. Drugs. 1988: 36: 429-454.

Hirata, D., et al. Saccharomyces cerevisiae YDR1, which encodes a member of the ATP-binding cassette (ABC) superfamily, is required for multidrug resistance. Curr Genet. 1994 Oct; 26(4): 285-94.

Hoeg, JM., et al. 3-Hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors in the treatment of hypercholesterolemia. JAMA. 1987 Dec 25; 248(24): 3532-6.

Holmes, M., et al. Improved shuttle vectors for Haloferax volcanii including a dual-resistance plasmid. Gene. 1994 Aug 19; 146(1): 117-21.

Horn, M. Coadministration of itraconazole with hypolipidemic agents may induce rhabdomyolysis in healthy individuals [letter]. Arch Dermatol. 1996 Oct; 132(10): 1254.

Horsman, Y., et al. Effects of simvastatin and pravastatin on 6 beta-hydroxycortisol excretion, a potential marker of cytochrome P-450 3A. Pharmacol Res. 1993 Oct-Nov; 28(3): 243-8.

Jakobisiak, M., et al. Cell cycle-specific effects of lovastatin, Proc Natl Acad Sci USA. 1991; 88: 3628-3632.

Jokubaitis, LA. Development and pharmacology of fluvastatin. Br J Clin Pract Symp Suppl. 1996 Jan; 77A: 11-5.

Joles, J., et al. Subcutaneous administration of HMG-CoA reductase inhibitors in hyperlipidaemic and normal rats. Lab Anim. 1992 Oct; 26(4): 269-80.

Jolley, KA., et al. Dihydrolipoamide dehydrogenase from the halophilic archaeon Haloferax volcanii: homologous overexpression of the cloned gene. J Bacteriol. 1996 June; 178(11): 3044-8.

Keesler, GA., et al. Stimulation by heme of steryl ester synthase and aerobic sterol exclusion in the yeast Saccharomyces cerevisiae. Arch Biochem Biophys. 1992 Aug 1; 296(2): 474-81.

Koga, T., et al. Tissue-selective inhibition of sterol synthesis in mice by pravastatin sodium after a single or repeated oral administrations. Lipids. 1995 Aug; 30(8): 775-9.

Koga, T., et al. Tissue selectivity of pravastatin sodium lovastatin and simvastatin. The relationship between inhibition of de novo sterol synthesis and active drug concentrations in the liver, spleen and testis in rat. Eur J Biochem. 1992 Oct , 1; 209(1): 315-9.

Kornkbrust, DJ., et al. Toxicity of the HGM-Co A reductase inhibitor lovastatin to rabbits. J Pharmacol Exp Ther. 1990. 1989. 348: 498-505.

Krause, BR., et al. Lipid-lowering activity of atorvastatin and lovastatin in rodent species: triglyceride-lowering in rats correlates with efficacy in LDL animals models. Atherosclerosis. Oct 1995; 117(2): 237-44.

Lam, WL., et al. Mevinolin-resistant mutatants identify a promoter and the gene for a eukaryote-like 3-hydroxy-3-methylglutaryl-coenzyme A reductase in the archaebacterium Haloferax volcanii. J Biol Chem. 1992 Mar 25; 267(9): 5829-34.

MacDonald JS., et al Preclinical evaluation of lovastatin. Am J Cardiol. 1988; 62: 16J-27J.

Mauro, VF. Clinical pharmacokinetics and practical applications of simvastatin [published erratum appears in Clin Pharmacokinet 1993 Jun; 24(6):518]. Clin Pharmacokinet. 1993 Mar; 24(3): 195-202.

Mayer, RJ., et al. Inhibition of 3-hydroxy-3-methylglutaryl coenzyme A synthase by antibiotic 1233A and other beta-lactones. Biochem Biophys Res Commun. 1990 Jun 15; 169(2): 610-6.

McCune, SA., et al. Effect of mevinolin on cholesterol metabolism in obese and lean Zucker rats. Biochem Pharmacol. 1987 Mar 15; 36(6). 875-9.

McKenney, JM. Lovastatin: a new cholesterol-lowering agent. Clin Pharm. 1988 Jan; 7(1): 21-36.

McPherson, R., et al. Effects of lovastatin on natural killer cell function and other immunological parameters in man. J Clin Immunol. 1993 Nov; 13(6): 439-44.

Miettinen, TA. Cholesterol metabolism during ketoconazole treatment in man. J Lipid Res. 1988 Jan; 29(1): 43-51.

Mitchell, JC., et al. Effects of lovastatin on biliary lipid secretion and bile acid metabolism in humans. J Lipid Res. 1991 Jan; 32(1): 71-8.

Mitsuzawa, H., et al. In vivo assays for farnesyltransferase inhibitors with Saccharomyces cerevisiae. Methods Enzymol. 1995; 250: 43-51.

Muench, TM., et al. Visceral mycosis in Chinook salmon (Oncorhynchus tschawytscha) due to Sporobolomyces salmonicolor. 1996 Mar; 33(2): 238-41.

Myre, SA., et al. Critical ketoconazole dosage range for ciclosporin clearance inhibition in the dog. Pharmacology. 1991; 43(5): 233-41.

Ng, WL., et al. Minimal replication origin of the 200-kilobase Halobacterium plasmid pNRC100. J Bacteriol. 1993 Aug; 175(15): 4584-96.

Nicholas, RO., et al. Correlation of inhibition of sterol synthesis with growth-inhibitory action. J Antimicrob Chemother. 1989; 23: 7-19.

Nucci, M , et al. Fungal infections in neutropenic patients. A 8-year prospective study. Rev Inst Med Trop Sao Paulo. 1995 Sep-Oct; 37(5): 397-406.

O'Donnell, MP., et al. Lovastatin but not enalapril reduces glomerular injury in Dahl salt-sensitive rats. Hypertension. 1992 Nov; 20(5): 651-8.

Pan, HY., et al. Comparative pharmacokinetics and pharmacodynamics of pravastatin and lovastatin. J Clin Pharmacol. 1990 Dec; 30(12): 1128-35.

Pan, HY. Clinical pharmacology of pravastatin, a selective inhibitor of HMG-CoA reductase. Eur J Clin Pharmacol. 1991; 40 Suppl 1: S15-8.

Pentikainen, PJ., et al. Comparative pharmacokinetics of lovastatin, simvastatin and pravastatin in humans. J Clin Pharmacol. 1992 Feb; 32(2): 136-40

Plazas, J., et al. Sporobolomyces salmonicolor lymphadenitis in an AIDS patient. Pathogen or passenger? [letter]. AIDS. 1994 Mar; 8(3): 387-8.

Quain, DE., et al. The effects of catabolite repression on the accumulation of steryl esters and the activity of β-hydroxymethylglutaryl-CoA reductase in Saccharomyces cerevisiae. J of General Microbiology. 1979. 111: 343-351.

Quion, JA , et al. Clinical pharmacokinetics of pravastatin. Clin Pharmacokinet. 1994 Aug; 27(2): 94-103.

Regazzi, MB., et al. Clinical efficacy and pharmacokinetics of HMG-CoA reductase inhibitors in heart transplant patients treated with cyclosporin A. Transplant Proc. 1994 Oct; 26(5): 2644-5.

Ryder, NS. Terbinafine: mode of action and properties of the squalene epoxide inhibition. Br J Dermatol. 1992 Feb; 126 suppl 39:2-7.

Sarajuddin, AT., et al. Relative lipophilicities, solubilities, and structure-pharmacological considerations of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors pravastatin, lovastatin, mevastatin, and simvastatin.J Pharm Sci. 1991 Sep; 80(9): 830-4.

Schuetz, DT., et al. Regulation of human liver cytochromes P-450 in family 3A in primary and continuous culture of human hepatocytes. Hepatology. 1993 Nov; 18(5): 1254-62.

Segaert, MF., et al. Drug-interaction-induced rhabdomyolysis. Nephrol Dial Transplant. 1996 Sep; 11(9): 1846-7.

Serizawa, N., et al. A two component-type cytochrome P-450 monooxygenase system in a prokaryote that catalyzes hydroxylation of ML-236B to pravastatin, a tissue-selective inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase. Biochim Biophys Acta. 1991 June 19; 1084(1): 35-40.

Servouse, M., et al. Isolation and characterization of yeast mutants blocked in mevalonic acid formation. Biochem Biophys Res Comm. 1984; 123: 424-430.

Sviridov, DD., et al. Comparison of the effect of six compactin-related compounds on cholesterol synthesis in five human cell types. Lipids, 1990 Nov; 25(11): 685-90.

Sheu, MJ., et al. Rhodotorula septicemia: report of a case. J Formos Med Assoc. 1994 Jul; 93(7): 645-7.

Shiao, MS., et al. Biosynthesis of mevinolin, a hypocholesterolemic fungal metabolite, in Aspergillus terreus. Proc Natl Sci Counc Repub China B. 1987 Jul; 11(3): 223-31.

Sirtori, CR. Tissue selectivity of hydroxymethylglutaryl coenzyme A (HMG CoA) reductase inhibitors. Pharmacol Ther. 1993 Dec; 60(3): 431-59.

Slater, EE., et al. Mechanism of action and biological profile of HMG CoA reductase inhibitors A new therapeutic alternative. Drugs. 1988; 36 Supl 3: 72-82

Treiber, LR., et al. Origin of monacolin L from Aspergillus terreus cultures. J Antibiot Tokyo 1989 Jan; 42(1): 30-6

Urbina, JA., et al. Antiproliferative synergism of the allylamine SF 86-327 and ketoconazole on epimastigotes and amastigotes of Trypanosoma (Schizotrypanum) cruzi. Antimicrob Agents Chemother. 1988; 32: 1237-1242.

Urbina, JA., et al. In vitro and in vivo synergism of binary and ternary combinations of ergosterol biosynthesis inhibitors as antiproliferative agents against Trypanosoma cruzi: therapeutic implications. Mem Inst Oswaldo Cruz 1991; 86(Suppl 1):33-35.

Vanden-Bossche, H. Biochemical targets for antifungal azole derivatives: hypothesis on the mode of action. Curr Top Med Mycol. 1985; 1: 313-51.

Vanderplasschen, A., et al. Madin Darby bovine kidney cell synchronization by lovastatin: application to bovine herpesvirus-1 gene expression. Vet Res. 1994; 25(6): 555-67.

VandeWaa, EA., et al. HMG-CoA reductase: role in schistosome egg production. IN: Molecular Strategies of Parasite Invasion, edited by N Agabian. New York: Liss. 1987; 527-40.

Vickers, S., et al. Metabolic disposition studies on simvastatin, a cholesterol-lowering prodrug. Drug Metab Dispos. 1990 Mar-Apr; 18(2): 138-45.

Vickers, S., et al. In vitro and in vivo biotransformation of simvastatin, and inhibitor of HMG CoA reductase. Drug Metab Dispos. 1990 Jul-Aug; 18(4): 476-83.

Vyas, KP., et al. Biotransformation of lovastatin--III. Effect of cimetidine and famotidine on in vitro metabolism of lovastatin by rat and human liver microsomes. Biochem Pharmacol. 1990 Jan 1; 39(1): 67-73.

Watkins, PB. The role of cytochromes P-450 in cyclosporine metabolism. J Am Acad Dermatol. 1990 Dec; 23(6 Pt 2): 1301-9; Discussion 1309-11.

Walsh, TJ., et al. Fungemia in children infected with the human immunodeficiency virus: new epidemiologic patterns, emerging pathogens, and improved outcome with antifungal therapy. Clin Infect Dis. 1995 Apr; 20(4): 900-6.

Watanabe, I., et al. Cloning, characterization and expression of the gene encoding cytochrome P-450sca-2 from Streptomyces carbophilus involved in production of pravastatin, a specific HMG-CoA reductase inhibitor. Gene. 1995 Sep 22; 163(1): 81-5.

Yamauchi, S., et al. Increase in serum and bile cholesterol and HMG-CoA reductase by lovastatin in Rats. Am J Physiol. 1991 Apr; 260(4 Pt 1): G625-30.

Zhou, LX., et al. Pharmacokinetic interaction between isradipine and lovastatin in normal, female and male volunteers. J Pharmacol Exp Ther. 1995 April; 273(1): 121-7.

cc: David Feigal
Director
Office of Drug Evaluation IV

Attachment 3

FISH & RICHARDSON P.C.

1425 K STREET, N.W.
11TH FLOOR
WASHINGTON, DC 20005

Telephone
202 783-5070

Facsimile
202 783-2331

Web Site
www.fr.com

June 13, 2003

Frederick P. Fish
1855-1930

W.K. Richardson
1859-1951

Dockets Management Branch
Food and Drug Administration
Department of Health and Human Services
Room 1061
5630 Fishers Lane
Rockville, MD 20852

Citizen Petition

BOSTON
DALLAS
DELAWARE
NEW YORK
SAN DIEGO
SILICON VALLEY
TWIN CITIES
WASHINGTON, DC

The undersigned submits this petition under 21 C.F.R. §10.25(a) and §10.30, to request the Commissioner of Food and Drugs to reclassify cyclosporine ("CSA") as a non-antibiotic drug and to remove it from the proposed list of drugs[1] that are ineligible for marketing exclusivity and patent listing pursuant to Section 125(d) of the Food and Drug Administration Modernization Act of 1997 ("FDAMA").[2] In the alternative, the undersigned requests the Commissioner to find that Restasis® is not an antibiotic drug product which falls under the ineligibility provisions of Section 125(d) and to grant Restasis® three year marketing exclusivity and patent listing rights pursuant to Section 505 of the Food Drug & Cosmetic Act ("FDCA").[3]

A. Action Requested

Petitioner Allergan Inc. is the holder of an approved new drug application ("NDA") for Restasis® Ophthalmic Emulsion, 0.05%, an ophthalmic formulation which includes the active ingredient CSA and is indicated for the treatment of "dry eye disease" in humans.[4] Historically, CSA and all drug products containing CSA were regulated as antibiotics under the FDCA despite the fact that CSA

[1] *See* Marketing Exclusivity and Patent Provisions for Certain Antibiotic Drugs, 65 Fed. Reg. 3223-02, Notice 99N-3088, proposed January 4, 2000 (to be codified at 21 C.F.R. pt. 314) ("Proposed Rule").
[2] Pub. L. No. 105-115, 111 Stat. 2296 (1997)
[3] Unless otherwise indicated, all references to the FDCA will be to sections of the Act rather than to sections of the U.S.Code.

exhibits no proven antibiotic properties and has never been approved or labeled for any antibiotic use.

Before 1997, new antibiotic drugs were regulated under Section 507 of the FDCA. In 1997, Congress repealed Section 507, moved antibiotic drug regulation under Section 505 and declared certain pre-FDAMA antibiotic drugs ineligible for various Hatch-Waxman benefits[5] including marketing exclusivity and Orange Book patent listing. In 1998, FDA developed a Guidance Document for Reviewers to explain the regulatory treatment of antibiotics following the repeal of Section 507.[6] In January 2000, FDA proposed new regulations to implement the repeal amendments ("Proposed Rule").[7] These regulations contain a list of antibiotic drugs ("exclusion list"), including CSA, that are ineligible for Hatch Waxman benefits. Under the FDA's Guidance and Proposed Rule, no NDA containing an active moiety of any drug on the proposed exclusion list is eligible for Hatch-Waxman benefits.

Allergan began development of Restasis® in September 1994, when it took over an Investigational New Drug ("IND") application then held by Sandoz. On February 24, 1999, Allergan filed its NDA 21-023 for Restasis®. Allergan received approvable letters from FDA on August 3, 1999, March 25, 2000 and October 19, 2002; on December 23, 2002, Restasis® was approved pursuant to Section 505. On March 3, 2003, FDA notified Allergan, by letter, of its Guidance Document and Proposed Rule dealing with the repeal of Section 507. In that

---

[4] The approved drug product is an ophthalmic emulsion of cyclosporine 0.05%, glycerin, castor oil, polysorbate 80, carbomer 1342 and sodium hydroxide to adjust the pH.

[5] Unless otherwise indicated, the term "Hatch-Waxman benefits" as used throughout this document means the marketing exclusivity, patent listing and patent certification benefits made available to pioneer drug manufacturers under Section 505.

[6] FDA's Guidance Document states that it "does not create or confer any rights on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the applicable statute, regulations or both." *See* GUIDANCE FOR INDUSTRY AND REVIEWERS: REPEAL OF SECTION 507 OF THE FEDERAL FOOD, DRUG, AND COSMETIC ACT, U.S. DEP'T. OF HEALTH AND HUMAN SERV., FOOD AND DRUG ADMIN. 1 fn 1 (1998).

[7] These regulations have never been adopted. *See* fn 1.

letter, FDA stated it was reassigning the Restasis® NDA 21-023 to an antibiotic application under NDA 50-790. Although Restasis® was not approved or labeled for any antibiotic indication, FDA refused to grant three year exclusivity or to accept patent information for Orange Book listing because Restasis® contains CSA, a drug on the FDA's exclusion list. As a result, Allergan currently has no protection under Hatch-Waxman against generic versions of Restasis® which could be approved at any time.

Allergan asserts that FDA's refusal to grant Hatch-Waxman protection to Restasis® is contrary to the FDCA and FDAMA and requests, therefore, that the following actions be taken immediately:

1. Removal of CSA from the proposed antibiotic exclusion list; and

2. Listing of Restasis® in the Orange Book for three years of marketing exclusivity as originally planned by FDA along with any patents which claim Restasis® or methods of using Restasis®.[8]

B. Statement of Grounds

CSA is not an antibiotic and, in fact, functions quite differently than an antibiotic. As explained further below, CSA should be removed from the FDA's antibiotic exclusion list for three reasons: (1) CSA was never approved by FDA as an antibiotic or labeled for any antibiotic indications; (2) CSA was initially, and mistakenly, classified as an antibiotic drug due solely to the literal reading of an overbroad definition; and (3) the 1997 FDAMA repeal amendments, which preclude marketing exclusivity for certain antibiotic drugs, were never intended to apply to drugs that were approved by FDA under 505 and for non-antibiotic indications. For these reasons, the inclusion of CSA on the FDA's proposed antibiotic exclusion list is both arbitrary and capricious.

---

[8] Patents which claim Restasis® or methods of using the drug are U.S. Pat. Nos. 4,649,047, 4,839,342 and 5,474,979.

Public policy also favors the removal of CSA from the exclusion list. By maintaining the improper classification of CSA as an antibiotic, new uses for this drug will not be pursued. Manufacturers will invest neither the time nor the resources to discover new indications for CSA if they cannot be assured of recovering their investments under the marketing exclusivity protections of the FDCA. When Allergan first began clinical studies on new indications for CSA, it understood that such indications would be eligible for Hatch-Waxman benefits under Section 505. Nothing in the legislative history of FDAMA remotely suggested to Allergan that such benefits were intended to be repealed. Moreover, Allergan relied, to its current detriment, on representations by FDA over a 10 year period that Restasis® was not an antibiotic drug and that exclusivity would be awarded. CSA and Restasis®, therefore, must be accorded the same Hatch-Waxman benefits available to other drugs regulated under Section 505.

Finally, despite CSA being on FDA's proposed exclusion list, Restasis® cannot be considered an "antibiotic drug" within the meaning of Section 125 of FDAMA. Restasis® was not the subject of an application for marketing received by the FDA under Section 507 prior to FDAMA. Accordingly, Restasis® is eligible to receive the Hatch-Waxman benefits accorded new antibiotic drug products regulated under Section 505.

1. Regulatory Background

Traditionally, the FDA approved non-antibiotic drugs pursuant to Section 505 and antibiotic drugs pursuant to Section 507. Prior to the 1984 Hatch-Waxman amendments, generic copies of non-antibiotic drugs were required to undergo the same level of clinical testing on safety and efficacy as required for pioneer drugs. For this reason, few non-antibiotic generics were approved before 1984. In the case of antibiotics, however, FDA routinely approved generic versions under Section 507 pursuant to monographs that were established

following initial drug approval. Thus, generic copies of antibiotics were not required to undergo lengthy and expensive clinical trials in order to obtain FDA approval. It was sufficient to show that they were identical to the chemical compound described in the pioneer drug monograph.

Hatch-Waxman changed the way non-antibiotic drugs were approved. Beginning in 1984, generic manufacturers were permitted to rely on the clinical data and other information submitted by the pioneer drug manufacturer and, as long as "bioequivalency" could be shown, the generic drug would be deemed safe and effective. In essence, Hatch-Waxman minimized many of the traditional distinctions between the two types of drug approval procedures. One other procedural distinction that previously existed was the requirement for batch certification of antibiotic drugs; however, this difference was also eliminated by regulations adopted in 1982, which exempted all antibiotics from batch certification.[9]

The 1982 regulations and 1984 amendments to the FDCA resulted in antibiotic and non-antibiotic drugs being treated in a very similar fashion.[10] Nonetheless, some important differences continued to exist in terms of the benefits available to drug manufacturers. One such benefit was five-year exclusivity under Section 505. Section 507(e) contained a "transfer" provision that required any antibiotic drug exempted from batch certification to be regulated under Section 505 following initial approval under Section 507.[11] This meant that an antibiotic drug would not be eligible for any of the Section 505 Hatch-Waxman benefits until after it was initially approved and exempted from batch certification. The effect of the transfer provision was to deny pioneer antibiotic drugs the five-year exclusivity rights that Section 505 grants to all pioneer non-antibiotic drugs. Nonetheless, three-year exclusivity was available under Section

---

[9] *See* 21 C.F.R. §433.1(1982).
[10] *See Glaxo v. Heckler* 623 F.Supp. 69 (E.D.NC 1985) ("*Glaxo I*").
[11] *See Glaxo v. Bowen*, 640 F.Supp. 933 (E.D. NC 1986)("*Glaxo II*").

505 for subsequent drug approvals (e.g. for new indications) as long as the antibiotic NDA contained clinical data supporting safety and efficacy.[12]

Following the Hatch-Waxman amendments in 1984, an antibiotic that was initially approved under Section 507 and exempted from batch certification was regulated identically to, and under the same statutory provisions as, a non-antibiotic drug. Indeed, many antibiotics such as CSA were regulated in this manner until the 1997. In that year, Congress enacted FDAMA, which, among other things, repealed Section 507 and placed all remaining antibiotic drug regulation[13] under Section 505. Congress' reason for doing this was to make five-year exclusivity available for pioneer antibiotic drugs to stimulate new research and investment.[14] The repeal amendment, set forth in Section 125(d) of FDAMA, also contained specific exclusionary language to ensure that antibiotic drugs that already had been the subject of industry research (i.e. approved antibiotics and Section 507 applications "received" by FDA prior to FDAMA) would not benefit from this new grant of exclusivity. Subsequently, FDA proposed regulations to implement the repeal of Section 507 and compiled a list of antibiotic drugs (including CSA) which would be subject to the Section 125(d)(2) exclusionary rules. FDA also proposed that any NDA submitted after 1997 that contains an antibiotic on the exclusion list would not be eligible for Hatch-Waxman benefits.

2. The Definition of "Antibiotic Drugs" was not Meant to Include CSA

CSA has never been approved by the FDA or labeled for any antibiotic indications and should not be considered an antibiotic drug under the law. Because no manufacturer has ever sought an antibiotic indication for CSA or submitted data to FDA showing CSA to be safe and effective as an antibiotic

[12] *Id. See* FDCA §§ 505(c)(3)(D) and 505(j)(3)(D).
[13] Pioneer antibiotic approvals and antibiotics not exempt from batch certification were then still regulated under Section 507.

agent, it should never have been regulated under Section 507. This historical oversight by FDA is an insufficient basis for denying Hatch-Waxman benefits for new drug products that provide new uses of CSA.

CSA was first approved by FDA in 1983 and regulated under Section 507 pursuant to the following antibiotic drug definition[15]:

> "antibiotic drug" means any drug intended for use by man containing any quantity of any chemical substance which is produced by a micro-organism and which has the capacity to inhibit or destroy micro-organisms in dilute solution (including the chemically synthesized equivalent of any such substance).

What is striking about this definition is its overbreadth. Applied literally, it encompasses products that are neither approved nor marketed for antibiotic indications. Indeed, it includes any drug product that contains even the smallest amount of any chemical substance produced by any microorganism as long as the substance has the capacity to inhibit or destroy any other microorganisms in a dilute solution. It does not matter how therapeutically ineffective such drug substance might be as an antibiotic nor how miniscule the drug's capacity for inhibiting other microorganisms. Moreover, the definition provides no guidance on what is meant by the term "inhibit" or what constitutes a "dilute solution." As a result, the statute's overbroad language forces upon FDA and drug manufacturers a regulatory scheme that may, in fact, have nothing whatsoever to do with any antibiotic therapy -- an outcome plainly at odds with what Congress intended when it adopted Section 507. [16]

Common sense dictates that any drug approved and regulated by FDA as an antibiotic must include the following essential elements: the drug must exhibit

---

[14] House Rep. No. 105-310, 105th Cong., 1st Sess. 77(1997).
[15] Section 507 contains essentially the same definition now found in Section 201(jj)
[16] Congressional intent for defining antibiotics under Section 507 was to encourage the development of antibiotic drugs by standardizing the approval process for this important class of chemical entities. At the time, Congress was unaware that the ultimate definition would prove to be overbroad and would include new technologies including drugs produced using recombinant DNA technologies.

at least some therapeutic properties of an antibiotic; it must contain at least one approved antibiotic indication; and it must be labeled and marketed as an antibiotic. Absent such essential elements, FDA would be forced to apply the definition to a host of drugs that are produced by micro-organisms but which are not thought to be, nor are regulated as, antibiotics.[17] For example, under a literal reading of the statute any drug produced by recombinant DNA technology would have to be tested for its capacity to inhibit micro-organism growth in a dilute solution and, if found to satisfy this requirement, would have to be approved as an antibiotic regardless of the indications being sought.[18] Many drugs approved as biologics would also have to be evaluated in this same fashion. Yet many such drugs are routinely approved by FDA under the non-antibiotic drug provisions of Section 505 and under the biologic provisions of the Public Health Service Act.[19] What this indicates is FDA uses additional screening criteria when determining whether a particular drug should be classified as an antibiotic and made to undergo the antibiotic approval process.

One obvious criterion is whether the drug manufacturer is seeking to have its drug labeled for antibiotic indications. In the examples cited (e.g. recombinant DNA and biologics), the drugs were obviously not seeking antibiotic labeling and thus, were approved under non-antibiotic provisions in the law. Applying the same criterion to CSA, once it was clear that CSA was not being approved for any antibiotic indications it should never have been classified as an antibiotic and regulated under Section 507.

---

[17] For an interesting list of possible drugs that may qualify see the pre-FDAMA drugs in the list of Approved Biotechnology Drugs 1999 *available at* http://www.bio.org/aboutbio/guide2.html (last visited May 28, 2003).

[18] By definition, a drug produced by recombinant DNA technology is produced by a microorganism, and thus should have been tested for inhibitory effect. Examples of such drugs are non-antibiotic approved drugs such as insulin, human growth hormone, other hormones, alglucerase, cladribine etc, and a host of biologics approved chemical entities including interferons, interleukins, erythropoeitin, streptokinase, etc.

[19] Public Health Service Act, Pub. L. No. 107-377, 58 Stat. 682 (codified as amended at 42 U.S.C. §§ 201-300hh-11 (2002)).

FDA cannot be allowed to arbitrarily pick and choose how it wants to classify drugs in isolation from the rest of the FDCA. To ignore the FDCA's overarching regulatory scheme of safety and effectiveness, in deference to an overbroad definition that is inconsistently applied, is to regulate in an arbitrary and capricious manner in violation of Constitutional requirements.[20] FDA must apply its drug classification regulations consistent with how drugs are approved and labeled. In such event, neither CSA nor Restasis® should be classified as an antibiotic drug under the FDCA.

3. CSA was Initially Regulated Under Section 507 by Mistake.

In 1957, a program was set up at Sandoz Ltd. whereby employees on business trips and vacations would gather soil samples as part of the search for new antibiotics from fungal metabolites.[21] In 1970, the fungus *Tolypocladium inflatum*[22] was isolated from two soil samples. Sandoz then set up a rigorous screening program that identified unknown metabolites from samples of fungi and tested them through a series of 50 pharmacological tests. Based on such testing, CSA was shown to have very weak inhibition of growth for a very select group of fungi and was virtually abandoned by Sandoz because of its lack of antibiotic activity. Eventually, however, CSA was revived when it was also

---

[20] A statute should not be read in isolation. *FDA v. Brown & Williamson*, 120 S.Ct.1291 (2000). Rather, the words of the statute must be read in their context with a view to their place in the overall statutory scheme. *Id* at 1301 (quoting *Davis v. Michigan Dept of Treasury*, 489 U.S. 803 (1989)). The statutory definition of antibiotic drug, if read in isolation from the rest of the FDCA or applied out of context with the rest of the statutory language, can result in a regulatory taking. *See Kolender v. Lawson*, 461 U.S. 352, 357 (1983) (holding that to be Constitutional a statute must not lend itself to arbitrary enforcement).

[21] The historical information in this section is all taken from an excellent discussion of the history of the development of cyclosporin that is available online as Harriet Upton, *Origin of Drugs in Current Use: The Cyclosporin Story, available at* http://www.oldkingdom.org/UG_projects/Harriet_Upton/Harriet_Upton.htm (last visited 03/27/2003). *See also* Karl Heusler and Alfred Pletscher, *The Controversial Early History of Cyclosporine*, 131 SWISS MED. WKLY 299-302 (2001); J.F. Borel and Z.L. Kis, *The Discovery and Development of Cyclosporine (Sandimmune®)*, 23 TRANSPLANT PROC. 1867-74 (1991); and H.F. Stähelin, *The History of Cyclosporine A (Sandimmune®) Revisited: Another Point of View*, 52 EXPERIENTIA 5-13 (1996).

[22] Cyclosporine is now taken from other fungal sources, but the molecule is the same.

found to have strong immunosuppressive activity. After much debate and further study, CSA was approved in November 1983 for the prevention of transplant rejection.[23]

CSA has always functioned therapeutically as an immunomodulator. It suppresses the growth of T-cells by blocking a specific chemical pathway.[24] More specifically, it has been shown to block the signal in lymphocytes to produce IL-1, IL-2, IL-3, IL-4 and γ-interferon, which results in the suppression of T-cell proliferation. Hence, CSA is not an antibiotic. Antibiotics act to kill or inhibit the growth of bacteria or other organisms in a human host. When dealing with infections, the last thing one would want to do is suppress the immune system. Understood in this manner, CSA operates essentially as an anti-antibiotic. Given its immunosuppressive properties, a doctor would never prescribe CSA to combat infection. Moreover, it is unclear that such a treatment would be worthwhile even for a fungal infection involving one of the few fungi that CSA was shown to inhibit *in vitro*. In view of other available effective antifungal therapies, it would make little clinical sense to suppress the very system that is in need of bolstering; accordingly, CSA cannot be considered an antibiotic within any accepted scientific meaning of such term.

CSA was originally submitted to FDA and accepted as an antibiotic because it met the overbroad definition in Section 507 based on the early studies performed showing the weak inhibition of certain fungi. As noted, however, CSA was never submitted to FDA for any antibiotic indications of use. And because there was little difference in the approval processes for antibiotic and non-antibiotic drugs when CSA was first approved, no advantage was to be gained

---

[23] Since that time, CSA has also been approved for use against severe psoriasis and rheumatoid arthritis.
[24] Cyclosporine specifically blocks activation of the phosphorylase enzyme calcineurin, which affects the immune response cascade. *See* Alexander M. Marsland and Christopher E.M. Griffiths, *The Macrolide Immunosuppressants in Dermatology: Mechanisms of Action*, 12 EURO. J DERM. 6 (November-December 2002).

from one classification or another.[25] As a result, CSA was inadvertently classified and accepted as an antibiotic in 1983.

Since CSA's initial approval, three additional indications have been approved for CSA in different forms. These indications are severe psoriasis and rheumatoid arthritis, both approved in 1997, and for dry eye (Restasis®) approved in 2002. None of these indications are antibiotic in nature and each benefit from the immunomodulatory effects of CSA. Immunomodulators work exactly the opposite of antibiotics in that they have immunosuppressive effects and not antimicrobial effects found in antibiotics. Given the regulatory history of CSA including all of the approved indications for use, it is clear that CSA should be classified as a non-antibiotic drug. In this regard, the final arbiter of any drug's classification must be the approved indications for use or such classification scheme becomes meaningless and arbitrary. For FDA to continue denying CSA its proper classification as a non-antibiotic drug will be to compound a 20-year-old mistake; accordingly, FDA must remove CSA from the proposed exclusion list.

4. Allergan has Detrimentally Relied on FDA's Representations that CSA and Restasis® are not Antibiotic Drugs.

For over 10 years Allergan had been in discussions with FDA on the development of its CSA-containing drug, Restasis®, and not once, prior to NDA approval, did FDA ever indicate to Allergan that Restasis® should be regulated as an antibiotic. It was only after Allergan had expended more than $5 million on research, development and clinical trials that FDA suddenly and unexpectedly declared, after approval, that Restasis® was an antibiotic drug ineligible for Hatch-Waxman benefits. Allergan submits that it is patently unfair for FDA to reclassify Restasis® at such a late date, so as to deny it the important Hatch-

[25] *See Glaxo I*, fn 10 *supra*.

Waxman benefits to which Allergan assumed it was entitled and which are accorded to other drugs similarly approved under Section 505. Had Allergan known ahead of time that Restasis® would be without any protections against generic entry, it likely would not have risked the substantial investment required to develop the product.

Allergan first began discussing CSA drug development with the FDA on June 17, 1992, after being authorized by Sandoz, the holder of the original CSA NDA. Allergan held a pre-IND meeting with FDA on July 11, 1994, during which FDA requested Allergan to investigate any changes in conjunctival flora – before and after treatment -- to determine whether CSA's immunosuppressive properties might cause infections. There were no discussions whatsoever as to CSA having any antimicrobial effects. On September 29, 1994, Sandoz transferred its IND rights to Allergan.

On February 24, 1999, Allergan filed its NDA (No. 21-023) for Restasis® requesting five years of exclusivity and received approvable letters from FDA on August 3, 1999, March 25, 2000 and October 19, 2000. On December 23, 2002, Restasis® was approved. Seven days later, FDA's Project Manager (HFD-550) contacted Allergan to say that Allergan had made a mistake on its exclusivity request and would be eligible for three years of exclusivity rather than the five years originally requested. Allergan, at this time, fully expected that FDA was carrying out its administrative function typical of approved 505 applications and would file all submitted patents in the Orange Book and list the three years of exclusivity. On January 21, 2003, Allergan was again contacted by the Project Manager and this time was told that it would be receiving no exclusivity based on FDA's "proposed" regulations that classified CSA, and all drugs containing CSA, as antibiotics. On March 3, 2003, FDA reclassified Restasis® as an antibiotic and issued a new NDA number 50-790.

Based on this record, there can be little doubt that Allergan was mislead by FDA from the beginning as to the proper classification of Restasis®. The drug had been developed and submitted under the non-antibiotic provisions of Section 505[26] and both Allergan and FDA discussed the Hatch-Waxman benefits that would be available upon approval. Allergan relied in good faith and to its detriment on the various statements, instructions and other representations made by FDA that Restasis® was not being treated as an antibiotic drug. Had there been any cause to doubt, during the10 years of FDA oversight, that such classification might be incorrect Allergan would have immediately addressed and resolved the matter in order to protect its substantial investment in this new drug.

As matters now stand, generic versions of Restasis® can be inexpensively developed and routinely approved by FDA, at any time, putting Allergan's entire $5 million plus invested in Restasis® at risk. This is grossly unfair to Allergan and its stockholders who are forced to bear the cost of FDA's oversight. Under the circumstances, the proper course of action is for FDA to take corrective action by removing CSA from its proposed exclusion list and declaring Restasis® to be eligible for the Hatch-Waxman benefits under Section 505.[27] FDA has the

---

[26] Allergan's NDA, for example did not contain any microbiology data that is required for an antibiotic drug approval. *See* 21 C.F.R.§314.50(d)(4).

[27] Government agencies, like private corporations, have an obligation to conduct their affairs in a reasonably efficient manner. *See Potomac Elec. Power Co. v. ICC*, 702 F.2d 1026, 1034 (D.C.Cir.1983) (warning that "excessive delay saps the public confidence in an agency's ability to discharge its responsibilities"). An entity that chooses to indulge inefficiencies cannot expect to be granted special dispensations. If "[t]he mills of the bureaucrats grind slow," *United States v. Meyer*, 808 F.2d 912, 913 (1st Cir.1987), then the agency, having called the tune, must pay the piper. *See, e.g., United States v. Baus*, 834 F.2d 1114, 1123 (1st Cir.1987) (holding that the government "should not be allowed by words and inaction to lull a party into a false sense of security and then by an abrupt volte-face strip the party of its defenses"); *Cutler v. Hayes*, 818 F.2d 879, 896 (D.C.Cir.1987) (explaining that, when an administrative agency loiters, "the consequences of dilatoriness may be great"). *Texaco Puerto Rico Inc. v Dep't. of Consumer Affairs*, 60 F.3d 867, 879 (1995).

requisite authority under FDCA and FDAMA to take such action and, moreover, the equities in this matter compel that such corrective actions be taken.[28]

5. Restasis® is not an "Antibiotic Drug" within the meaning of Section 125 (d)(2) of FDAMA.

When Congress passed FDAMA in 1997, it repealed Section 507 specifically to make pioneer antibiotic drugs eligible for the Hatch-Waxman benefits.[29] Congress believed that five-year exclusivity was needed to increase industry "research toward the development of new antibiotics." Congress made clear that it wanted to stimulate new research, rather than to reward old research,[30] and thus, it was careful to limit the grant of new rights "to those products that are New Chemical Entities and to products for which a New Drug Application has not been submitted to FDA.[31]

Section 125(d) of FDAMA carried out this regulatory scheme. Subsection (d)(1) set forth the general rule that any antibiotic drug previously approved by FDA under Section 507 would, henceforth, be regarded as having been approved under Section 505 [32]; and subsection (d)(2) provided an "Exception" to the Hatch-Waxman benefits for any antibiotic drugs which were the subject of applications

---

[28] The FDAMA repeal amendment was directed to antibiotic drugs that were properly regulated under Section 507. A drug that was improperly or mistakenly regulated under Section 507 was never intended by Congress to be denied the Hatch-Waxman benefits under Section 505.

[29] In *Glaxo I*, a drug manufacturer argued that the transfer provision, in fact, conferred Section 505 marketing exclusivity on the new antibiotic drug as of the FDA application filing date thereby qualifying such drug for five years of marketing exclusivity. The district court disagreed with this reading of the statute, and held that "[o]nly following approval is an antibiotic drug then exempted and treated as a nonantibiotic by virtue of [the] transfer provision.

[30] Applications received by the FDA prior to FDAMA were, by definition, the subject of antibiotic research and development activities that had already been completed. Five-year exclusivity was not needed, therefore, to incentivize the pursuit of these applications.

[31] *See supra* fn 13 and accompanying text.

[32] By treating pre-FDAMA approvals as having been made under Section 505, Congress eliminated the possibility of the any approved drug, or active ingredient of any approved drug, becoming eligible for five-year exclusivity.

that had been filed under Section 507 and received by FDA prior to FDAMA.[33] Together these provisions brought all new antibiotic drug applications within the scope of Section 505 but without creating new rights in existing drug products.

In the January 2000 Proposed Rule implementing the Section 507 repeal, FDA interpreted Section 125(d)(2) in an unusual manner. It interpreted the amendment as actually denying Hatch-Waxman benefits for any antibiotic drug product – old or new – if the product's active moiety was previously the subject of an application received under Section 507. Under such interpretation, any antibiotic product regulated under Section 505 prior to FDAMA would no longer be eligible for Hatch-Waxman benefits pursuant to the Section 507(e) transfer provision.[34] Moreover, any Hatch-Waxman benefits, which were in existence at the time of FDAMA passage, would now be nullified. Such a reading of the repeal amendments, which comes perilously close to a legislative taking, finds no support anywhere in the public record. Indeed, the rare bit of legislative history that deals with Section 507 repeal comes from the House Report, which states that new grant of exclusivity was intended to *increase* drug research on new "products" – not just active moieties. Had Congress intended Section 125(d)(2) to limit Hatch-Waxman benefits to new active moieties rather than new antibiotic products, it presumably would have spoken clearly as it had in the 1984 amendments.[35]

---

[33] Section 125(d)(2) provides that various Hatch-Waxman rights shall not apply "to any application for marketing in which the drug that is the subject of the application contains an antibiotic drug and the antibiotic drug was the subject of any application for marketing received [by the FDA] under Section 507 ...before the date of [FDAMA]." (emphasis added).

[34] CSA was initially approved in 1983 and exempted from batch certification in the 1984 pursuant to an FDA monograph. Hence, any CSA-based drug product submitted to FDA with clinical trials, prior to the passage of FDAMA, would have been eligible for three-year marketing exclusivity and patent listing rights in the Orange Book under Section 505 and the holdings in *Glaxo I and II*.

[35] It must be presumed that Congress knew the difference between drugs and active moieties when it drafted Section 125. The original exclusivity provisions in the 1984 Hatch-Waxman Act referred to a drug's "active ingredients", a term that FDA found later to be synonymous with active moiety. Congress chose not to use the same term in its FDAMA amendments and FDA is required to give significance to such fact.

Instead, Congress specifically elected to use the term "antibiotic drug,"[36] which is defined broadly in the FDCA as "any drug containing any quantity of any chemical substance ...or any derivative thereof."[37] To determine what Congress meant by such term in the context of Section 125(d)(2), FDA chose not to look to the plain language in the statute but to the FDA's history of applying Hatch-Waxman exclusivity. It found that it had consistently looked at a drug's active moiety[38] in determining whether exclusivity protections should apply and concluded from this that the same test should be used for limiting the Hatch-Waxman benefits under the FDAMA repeal amendments.[39] But such analysis is flawed as it ignores the fact that in 1984, when marketing exclusivity was first introduced, Congress specifically directed the FDA to look to a drug product's active ingredient -- a term which FDA considers synonymous with active moiety -- when determining such rights. By comparison, the 1997 amendments do not contain a single reference to an antibiotic drug's active ingredient, a term with which Congress was long familiar. If anything then, FDA should have construed the term "antibiotic drug" to mean antibiotic drug product rather than antibiotic active moiety. Such interpretation would give effect to Congress' intent of encouraging research and development of new antibiotic products and would preserve the Hatch-Waxman benefits that were available, prior to FDAMA, to new antibiotic drug products like Restasis®.

Insofar as Restasis® is a drug product that was not the subject of any Section 507 marketing application "received" by FDA prior to FDAMA and was never developed as an antibiotic drug nor shown to have any antibiotic properties during its many years of development, it does not come within the exclusionary

---

[36] *See supra* fn. 15 and accompanying text.
[37] 21 U.S.C. § 321(jj) (200) (emphasis added).
[38] An active moiety is defined narrowly by FDA as "the molecule or ion responsible for the physiological or pharmacological action of the drug substance." 21 C.F.R. § 314.108 (a) (2002).
[39] *See* Proposed Rule at 3625.

language of Section 125(d)(2). Restasis®, therefore, is eligible for Hatch-Waxman benefits based on its Section 505 approval.

Conclusion

CSA should be removed from the FDA's exclusion list for the reasons stated. In any event, Restasis® is neither approved nor labeled for any antibiotic indications and, therefore, cannot be considered an antibiotic drug under the law. Restasis® must be given the full Hatch-Waxman benefits provided under Section 505. To deny such benefits represents a gross misreading of the 1997 FDAMA repeal amendments and will stifle industry research on new drug products in contravention of Congressional intent, public policy and the FDCA.

C. Environmental Impact

This petition is categorically excluded from the environmental impact statement requirement under 21 C.F.R. §25.31.

D. Economic Impact

The Commissioner has not requested any economic impact information at this time.

E. Certification

The undersigned certifies, that to the best of his knowledge and belief, this petition includes all information and views on which the petition relies, and that it includes representative data and information known to the petitioner, which are unfavorable to the petition.

Respectfully submitted,

Terry G. Mahn
John E. Mauk
Fish & Richardson P.C.
1425 K Street
11th Floor
Washington D.C. 20005

Counsel for Petitioner

cc: Christine F. Rogers

Attachment 4

FISH & RICHARDSON P.C., P.A.

3300 Dain Rauscher Plaza
60 South Sixth Street
Minneapolis, Minnesota
55402

Telephone
612 335-5070

Facsimile
612 288-9696

Web Site
www.fr.com

Frederick P. Fish
1855-1930

W.K. Richardson
1859-1951

BOSTON
DALLAS
DELAWARE
NEW YORK
SAN DIEGO
SILICON VALLEY
TWIN CITIES
WASHINGTON, DC

AUGUST 1, 2003

Dockets Management Branch
Food and Drug Administration (HFA-305)
Department of Health and Human Services
Room 1061
5630 Fishers Lane
Rockville, MD 20852

PETITION FOR STAY OF ACTION

The undersigned submits this Petition for Stay of Action under 21 C.F.R. § 10.35, on behalf of Allergan, Inc., requesting FDA to stay its approval of all Section 505(j) Abbreviated New Drug Applications ("ANDAs") and Section 505(b)(2) New Drug Applications for generic versions of Restasis®, Ophthalmic Emulsion 0.05%, pending disposition of Allergan's pending Citizen Petition in Docket No. 2003P-275/CP-1. In addition, Allergan requests that FDA immediately list Allergan's patents for Restasis® in the Orange Book. Allergan seeks a decision on this stay petition as soon as possible and no later than thirty days after it has been received by the FDA. Allergan will consider any failure to grant such relief in that period of time a final decision of the FDA for purposes of seeking judicial review.

A. Decision Involved

On June 13, 2003, Allergan filed a Citizen Petition requesting that it be accorded three years of market exclusivity along with Orange Book patent listing rights for Restasis® (NDA 21-023), approved on December 23, 2002, under Section 505 of the Food Drug & Cosmetic Act ("FDCA"). Allergan's Citizen Petition was necessitated by FDA's subsequent and improper reclassification, on March 3, 2003, of Restasis® as an antibiotic drug product (NDA 50-790). This reclassification occurred some three months after Restasis® was approved by FDA under Section

505, some ten years after development first began and after Allergan spent over $47 million dollars in Research and Development costs. By reclassifying Restasis® in this manner, FDA rendered the drug ineligible for Hatch-Waxman benefits pursuant to a proposed, but yet to be adopted, rule implementing Section 125(d) of the Food and Drug Administration Modernization Act of 1997 (FDAMA). FDA has not yet acted on Allergan's Citizen Petition.

B. Action Requested

FDA is requested to stay its approval of all ANDAs and Section 505(b)(2) applications for generic versions of Restasis® until it has ruled on Allergan's pending Citizen Petition and, if FDA denies that petition in whole or in part, until twenty days after that decision to permit Allergan to seek a judicial stay. Allergan believes that the need for a stay in this case is particularly compelling because of the streamlined regulations set forth in 21 C.F.R. § 320.22 (b) which apply to bioequivalency determinations for generic ophthalmic solutions. In particular, Section 320.22(b) requires that FDA "shall" waive the requirement for evidence of *in vivo* bioequivalency upon a showing that a generic ophthalmic solution contains the same active and inactive ingredients in the same concentration as the reference listed drug. Generic manufacturers of Restasis®, therefore, are in a position to receive rapid approval of their ANDAs and Section 505(b)(2) applications.[1] Without the right to list Restasis® patents in the Orange Book, Allergan will not receive any notice that generic applications have been submitted to FDA nor will it be able to take advantage of the thirty month stay provisions should patent litigation ensue. To avoid irreparable harm to Allergan, FDA is requested to adhere to its initial and correct classification and approval of Restasis® as a non-antibiotic drug product eligible for Hatch-Waxman benefits or, in the alternative, to find that Restasis® is a new

[1] In a companion filing to this Petition, Allergan is amending its Citizen Petition to provide evidence of its current U.S. investment in Restasis® -- a sum which exceeds $47 million.

antibiotic drug product that does not fall within the Hatch-Waxman ineligibility provisions of Section 125 of FDAMA.

In either event, Allergan further requests that FDA immediately list Allergan's patents for Restasis® in the Orange Book, at least until such time as the Citizen Petition has been decided and Allergan has an opportunity for judicial review of that decision. Accordingly, Allergan is resubmitting the patent information for Restasis® as Exhibit A to this petition. FDA improperly refused to list the patent information for this drug at the time of its approval. That listing should now occur, at least provisionally during the pendency of the requested stay. FDA's failure to grant Allergan patent listing rights along with the right to receive notice of generic drug applications and approvals under 21 U.S.C. §§ 355(b), (c), and (j) will prejudice Allergan's ability to enforce its patents pursuant to Section 271(e)(2) and protect its investment in Restasis®.

C. Statement Of Grounds

1. Mandatory Stay

Under 21 C.F.R. § 10.35(e), FDA must grant a stay of action if all of the following apply:

(a) the petitioner will otherwise suffer irreparable injury

(b) the petitioner's case is not frivolous and is being pursued in good faith;

(c) the petitioner has demonstrated sound public policy grounds supporting the stay; and

(d) the delay resulting from the stay is not outweighed by public health or other public interests.

As demonstrated below, all of these criteria are met.

a. Allergan will suffer irreparable injury

If this Petition for stay is denied by FDA and generic versions of Restasis® are approved and enter the market, it is axiomatic that Allergan will immediately lose significant sales and market share. Even if a court should subsequently overturn the FDA's denial of this Petition, Allergan will be unable to recoup such losses; thus, it will be irreparably harmed.

Such harm is not a remote possibility. Restasis® has been hailed as "the first prescription treatment that has been shown to help improve the quality and quantity of tears" for treating dry eye syndrome, a common ailment.[2] Absent a favorable ruling on the Citizen Petition and this Petition to Stay, Restasis® will not receive three years of market exclusivity and Allergan will not be given the opportunity to enforce its patents under Hatch-Waxman. Manufacturers of low cost generics will be able to cash in quickly on the tremendous market potential for this new drug, putting Allergan's investment of more than $47 million in Restasis® at risk. Because such losses can never be recovered once generic products enter the market, there can be little doubt that Allergan will be irreparably harmed by a denial of this Petition.[3]

b. Allergan's case is not frivolous and is being pursued in good faith

---

[2] Stefanie Weiss, *How Dry Eye Am*, Washington Post, July 1, 2003, at F5 (attached as Exhibit B). *See also* Lynda Charters, *Restasis Approval A Milestone For Dry Eye*, Ophthalmology Times, February 1, 2003, at 1 ("The FDA approval of cyclosporine ophthalmic emulsion 0.05% (Restasis, Allergan) Dec. 26 marked a landmark for ophthalmology. The eye drop therapy for moderate to severe keratoconjunctivitis sicca is unique in that it treats the inflammatory process that causes the condition, and not just its symptoms.") (attached as Exhibit C); Laurie Barber, M.D., *Clinical Experience with Cyclosporine (Restasis) for Dry Eye*, March 2003, available at http://www.eyetowncenter.com/eyetc/11.541/0.21/0.22/0.145/0.1/0.0/0.0/articles.htm ("There is considerable pent-up demand among dry eye patients who have simply given up on the medical profession.") (attached as Exhibit D); Michelle Stephenson, *The Flap's Important Role In LASIK-Induced Dry Eye/Restasis: Getting beyond the dry facts*, Eye World, July 2003 (available at http://www.eyeworld.org/july03/0703p36.html ("When Restasis (Allergan, Irvine, Calif.) gained Food and Drug Administration approval last December, for the first time ophthalmologists found that they were able to get at the underlying cause of dry eye disease rather than simply offering patients palliative options.") (attached as Exhibit E).

[3] *See CollaGenex Pharmaceuticals, Inc. v. Thompson*, CV 03-1405 (D.C.D.C. July 22, 2003), in which the court discusses the devastating impact of generic entry on pioneer drugs.

Allergan's Citizen Petition makes a compelling case for the relief requested. As explained in the Citizen Petition, Allergan is suffering the consequences of repeated FDA errors concerning the historic regulation of cyclosporine (CSA), the active ingredient in Restasis®.

FDA's first error occurred in 1983 when CSA was inappropriately classified as an antibiotic drug despite the fact that CSA does not function as an antibiotic and had never been approved for any antibiotic indications. In point of fact, CSA has been shown to be an immunosuppressive compound that functions essentially as an "anti-antibiotic."[4] For this reason, Restasis® is contraindicated for patients with eye infections -- conditions that are commonly treated with antibiotic drugs.[5]

Significantly, one court recently held that the FDA cannot classify a drug product as an antibiotic if, in fact, it exhibits no antibiotic properties. *See CollaGenex Pharmaceuticals, Inc. v. Thompson*, CV 03-1405 (D.C.D.C. July 22, 2003) (attached as Exhibit F). In *CollaGenex*, the district court enjoined FDA from approving any ANDAs for a generic version of Periostat® (doxycycline hyclate 20 mg) because, at the concentration of the active ingredient authorized, the drug product did not have the capacity to inhibit or kill microorganisms as required of an antibiotic drug under 21 U.S.C. § 321(jj). Similar to the situation here, CSA, in the concentration approved for Restasis® (0.05%), has never been shown to have <u>any</u> capacity to inhibit or kill microorganisms. Based on the holding in *CollaGenex*, Restasis® cannot be properly classified as an antibiotic drug.

At the time of FDA's decision in 1983, its consequences were minimal because antibiotic drugs were not then discriminated against for purposes of Hatch-

---

[4] As Allergan's Citizen Petition explains, an immunosuppressive reagent is essentially the opposite of an antibiotic, which inhibits or destroys microorganisms. In contrast, an immunosuppressive reagent enables microorganism growth because it suppresses the immune system by blocking activation of the phosphorylase enzyme calcineurin. *See* Citizen Petition at 10.

[5] *See* Restasis® product information sheet, available at www.restasis.com ("RESTASIS™ is contraindicated in patients with active ocular infections.").

Waxman as they are today. In any event, Allergan was not a party-in-interest to that early determination.

FDA's second error occurred in 2000 when it construed FDAMA's so-called "antibiotic repeal" provisions in a manner that penalizes pioneer drug manufacturers, contrary to Congressional design. As Allergan explains in its Citizen Petition, Section 125 of FDAMA was intended to stimulate research and investment in new antibiotic drugs by making pioneer antibiotics newly eligible for Hatch-Waxman benefits.[6] To avoid any unintended windfalls to manufacturers of "old" antibiotics, Congress placed restrictions on certain drug approvals. Thus, Section 125(d)(2) provides that any antibiotic drug that was "the subject of any application for marketing received [by FDA] under Section 507 . . . before [passage of FDAMA]" would be ineligible for Hatch-Waxman benefits (*e.g.*, market exclusivity, patent certification and Orange Book listing).[7]

Restasis®, however, had not previously been the subject of a Section 507 application received by FDA and, therefore, Allergan was operating under the clear assumption that FDAMA's "exception" to Hatch-Waxman had no applicability. Allergan's assumption squared with the statutory language, the clear Congressional intent and the public comments of several of the drafters.[8] Accordingly, Allergan had every reason to expect that Restasis® would be eligible for Hatch-Waxman benefits upon approval – an expectation that was confirmed by FDA's initial classification of Restasis® as a 20,000-series (non-antibiotic) application (NDA 21-023) in February 1999, and subsequent approval in December 2002.

---

[6] House Rep. No. 105-310, 105th Cong., 1st Sess. 77 (1997). Prior to 1997, antibiotics were regulated under Section 507 and thus, ineligible for Section 505 Hatch-Waxman benefits.

[7] This "exception" to Hatch-Waxman was in recognition of the fact that any antibiotic drug product that had been "received" by FDA prior to FDAMA was, by definition, one which already had been fully developed and clinically tested and therefore, was not in need of new "research and investment" which Hatch-Waxman was designed to stimulate.

[8] *See* letter from Rep. Tom Bliley, Chairman, House Commerce Committee, Rep. Michael Bilirakis, Chairman, House Commerce Subcommittee on Health and Environment, and Richard Burr, member of the House Commerce Committee to Michael A. Friedman, M.D., Lead Deputy Commissioner, U.S. FDA (May 21, 1998), reprinted in *FDA WEEK*, January 28, 2000

In January 2000, however, FDA released a "proposed rule" which construed Section 125(b)(2) as *denying* Hatch-Waxman benefits to any NDA containing an "active moiety" of any antibiotic drug that had ever been the subject of an application received under Section 507.[9] FDA prepared a list of such pre-FDAMA antibiotic drugs that included CSA. Under FDA's novel and arbitrary interpretation of Section 125, Restasis® would fall within the Hatch-Waxman exception if it were classified as an antibiotic drug product.

FDA's third and most recent error was its post-approval reclassification of Restasis® as an antibiotic drug product. After having already approved Restasis® as a 20,000-series nonantibiotic drug on December 23, 2002, after many years of treating Restasis® as an immunosuppressive drug for purposes of approval, FDA unexpectedly changed course and reclassified it as a 50,000-series antibiotic drug on March 3, 2003, making it ineligible for Hatch-Waxman benefits under FDA's enforcement of its proposed rule. Allergan relied on FDA's previous classification when it continued investing tens of millions of dollars into the research and development of Restasis®. FDA should therefore be estopped from changing course so late in the process. FDA's action unfairly denies Restasis® the Hatch-Waxman rights to three years of market exclusivity and Orange Book patent listing which are vital to its commercial success. For these reasons, Allergan's cause of action is non-frivolous and is being pursued in good faith.

c. Sound public policy grounds support the stay

Hatch-Waxman represents a carefully balanced compromise between pioneer and generic drug manufacturers. It is intended to encourage the costly research and development efforts that lead to the discovery of new drugs while, at the same time, expedite the availability of safe, effective, and less expensive versions of approved

[9] *See* Marketing Exclusivity and Patent Provisions for Certain Antibiotic Drugs, 65 Fed. Reg. 3623-02, Notice 99N-3088, proposed January 24, 2000 (Proposed Rule).

drugs. FDA's arbitrary classification of the immunosuppressive drugs CSA and Restasis® as antibiotic drugs not eligible for Hatch-Waxman benefits significantly deprives Allergan, as the NDA holder, of the benefits of the carefully crafted Hatch-Waxman bargain. Moreover, such improper classification confers a potential windfall on ANDA and 505(b)(2) applicants who are now in a position to obtain rapid approvals of generic versions of Restasis® based on Allergan's clinical data. Such windfall is especially unfair in the case of ophthalmic solutions where bioequivalency may be determined to be self-evident under 21 C.F.R. § 320.22. Because Hatch-Waxman benefits are critical to stimulating research and development of costly new drug products, any action which threatens the balance struck by Congress between pioneer drug manufacturers and generics also threatens the public interest. A stay in this case, therefore, is supported by sound policy goals.

d. Any delay will not harm the public interest

Allergan plans to seek court review if FDA denies its Citizen Petition or this Petition for Stay. Allergan anticipates that a court would view this case as raising significant public policy concerns and would decide the case quickly, minimizing the impact of any delay in generic approvals.

Indeed, Allergan is not the only company to have strongly disagreed with FDA's proposed rules interpreting of Hatch-Waxman's impact on antibiotic drugs. Several other drug manufacturers, as well as Pharmaceutical Research and Manufacturers of America ("PhRMA"), filed extensive comments on the FDA's proposed rule, challenging its unusual and arbitrary interpretation of FDAMA.[10]

---

[10] *See* Comment from PhRMA of April 24, 2000 (arguing that FDAMA applies only to antibiotic drug products, not active moieties) (attached as Exhibit G); Comment from SmithKline Beecham of April 14, 2000 (same) (attached as Exhibit H); Comment from Merck of April 21, 2000 (disagreeing with FDA's interpretation of "active moiety") (attached as Exhibit I); Comment from Alcon of April 21, 2000 (arguing that "old" antibiotics still receive Hatch-Waxman benefits under 35 U.S.C. § 271(e)(2)) (attached as Exhibit J), and Comment from AstraZeneca of January 24, 2001 (arguing that FDA improperly classified meropenem as an antibiotic, not an anti-infective agent) (attached as Exhibit K).

These comments provide powerful evidence that the legislative drafters of Section 125 did not intend to exclude new antibiotic drug products from receiving Hatch-Waxman benefits under Section 505.[11]

There is no public health benefit or other issue of public interest in sustaining arbitrary and capricious drug classifications that deprive NDA holders of their exclusivity and marketing rights under the applicable statutes and regulations. Nor is there any public interest in allowing approval of generic drugs under an illegitimate classification system. "The public's interest in 'the faithful application of the laws' outweigh[s] its interest in immediate access to [a competing] product." *Mova Pharmaceutical Corp. v. Shalala*, 140 F.3d 1060, 1066 (D.C. Cir. 1998).

2. Discretionary Stay

Finally, even if FDA finds that the criteria for a mandatory stay set forth above are not met, FDA may nevertheless grant a discretionary stay if it is "in the public interest and in the interest of justice." 21 C.F.R. § 10.35(e). The issues raised by Allergan's Citizen Petition are both novel and important. In *CollaGenex*, a case involving similar questions of drug classification, the pioneer drug manufacturer obtained a court-imposed stay much like Allergan is seeking. FDA, therefore, should grant this stay request pending resolution of these issues for all similarly situated manufacturers. Such issues are far from being settled, as evidenced by the pendency of FDA's three year old proposed rules dealing with antibiotic drug classifications, yet the FDA has proceeded to enforce those rules prematurely. The public interest and the interests of justice demand expeditious, certain, and even-handed resolution of the issues.

D. Conclusion

---

[11] *Id.*

Allergan's Citizen Petition asks that FDA remove CSA from the list of proposed antibiotics that are ineligible for marketing exclusivity and patent listing, or alternatively to find that Restasis® is not an antibiotic drug product. The FDA has erred in its classification of CSA as an antibiotic compound and its interpretation of FDAMA as excluding Restasis® from eligibility for Hatch-Waxman benefits. These errors have stripped away Allergan's rights to market exclusivity and Orange Book patent listing for Restasis® after an expenditure of over $47 million dollars in costs and years of reliance on FDA's previous position that the drug was not an antibiotic.

For the reasons provided herein, FDA should, within thirty days of this petition, grant a stay of approval of all ANDA and 505(b)(2) applications for generic forms of Restasis® pending a final determination on Allergan's pending Citizen Petition. In addition, at least until FDA makes a decision on the Citizen Petition, FDA should list the patents for Restasis® in the Orange Book to alleviate the current harm being done to Allergan under FDA's enforcement of its proposed rule. Should FDA ultimately deny the relief requested herein, Allergan asks that it be given sufficient time (at least twenty days) to seek a judicial stay before FDA approves any generic drug applications.

Respectfully submitted,

Wendy A. Vicente

Terry G. Mahn
Wendy S. Vicente
Fish & Richardson P.C.
1425 K Street
11th Floor
Washington, D.C. 20005

Counsel for Petitioner

Attachment 5

DECLARATION OF DIANE D-S. TANG-LIU, Ph.D.

Diane D-S. Tang-Liu, Ph.D. makes the following declaration:

1. I am Vice President of Pharmacokinetics and Drug Metabolism, Research and Development, at Allergan, Inc. I have worked at Allergan for the past twenty years in various capacities. Additionally, I am a Professor at the School of Pharmacy and Department of Pharmaceutical Sciences at the University of Southern California. A full statement of my education and professional accomplishments is contained in my curriculum vitae, which is attached as Exhibit A to this Declaration.

2. I have been asked to comment on the human tissue concentrations of cyclosporine A ("CsA") after recommended twice daily dosing of Restasis® (cyclosporine ophthalmic emulsion 0.05%) and in particular on the question of whether any such concentrations would reach the level of 0.1 micrograms per milliliter. Restasis® is a topical eye drop preparation of CsA used for the treatment of moderate and severe dry eye. It acts as an immunosuppressant, not as an anti-infective or as an antibiotic, and suppresses the immune system targeting the actual cause of dry eye disease. I have had extensive experience with the research, development, and FDA approval of Restasis®. I am the authority at Allergan on the pharmacokinetics of Restasis®, and have conducted experiments to measure blood concentrations of CsA after applying Restasis®. Furthermore, I have reviewed all of the relevant literature regarding the ocular

pharmacokinetics and ocular tissue levels of CsA after application to the ocular surface.

3. Human blood levels of CsA after twice daily administration of Restasis® are nondetectable. Allergan has attempted to detect blood CsA concentrations using a specific and sensitive high pressure liquid chromatography-mass spectrometry assay. Blood concentrations of CsA, in all of the specimens collected, after administration of Restasis® 0.05% to the ocular surface twice a day, in humans for up to twelve months, were below the quantitation limit of 0.1 nanograms per milliliter (i.e., 0.0001 micrograms per milliliter). Furthermore, there was no detectable drug accumulation in blood during the twelve months of treatment with Restasis®.

4. Unlike drawing blood from a patient's vein, sampling ocular tissues for the purpose of measuring CsA concentrations after administration to the ocular surface is difficult and in most cases, because ocular biopsy would create inappropriate risks in dry eye treatment, ethically prohibited. As a result, studies measuring CsA concentrations in human ocular tissues after the application of Restasis® are not available. Therefore, there is no evidence that the ocular tissue concentrations of CsA after twice-daily Restasis® would result in concentrations at or above 0.1 micrograms per milliliter in ocular tissues or any other human tissues of the body.

5. The CsA molecule is relatively large, and when applied to the surface of the eye, very little of the drug penetrates through the ocular surface. Based on my experience with the development of Restasis® and my review of all available data on the pharmacokinetics of cyclosporine applied to the ocular surface, I conclude that use of